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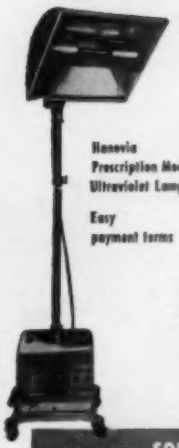

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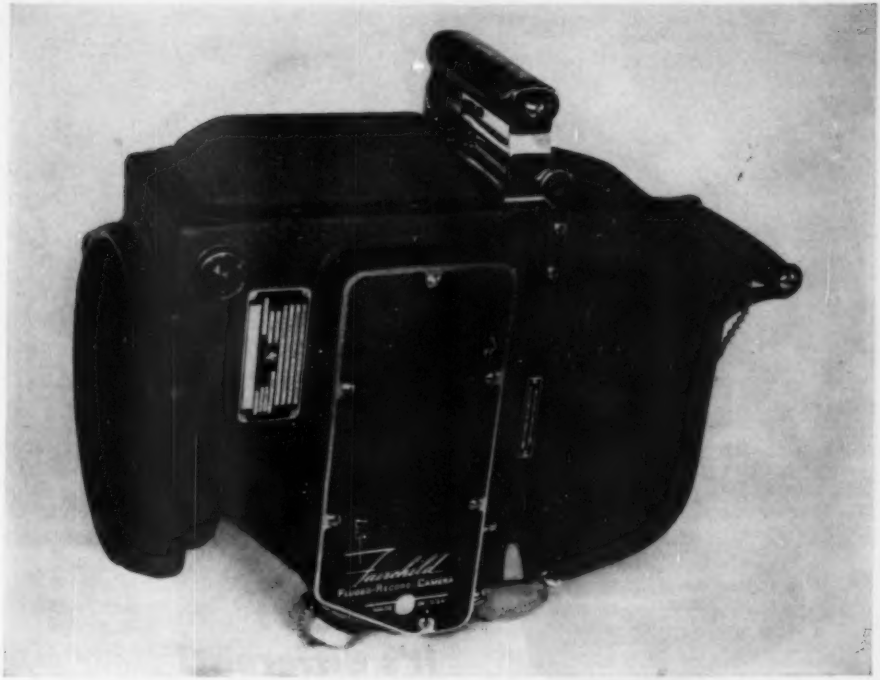

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CARDIOPULMONARY FUNCTION IN HEMATOGENOUS PULMONARY TUBERCULOSIS IN PATIENTS RECEIVING STREPTOMYCIN THERAPY[1, 2]

JOHN H. McCLEMENT,[3] ATTILIO D. RENZETTI, JR., DOUGLAS CARROLL, AARON HIMMELSTEIN, AND ANDRE COURNAND

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INTRODUCTION

Although dyspnea and cyanosis have been recognized for many years to be part of the clinical picture of acute hematogenous[4] tuberculosis, no study of cardiopulmonary physiology during the course of this disease has been published. It is the purpose of this paper to give a report on the state of cardiopulmonary function during the course of, and after recovery from, acute hematogenous tuberculosis.

It is well known that untreated acute generalized hematogenous tuberculosis is an almost uniformly and rapidly fatal disease. Pathologic studies on this early and untreated stage of the disease have shown (1) that in the earliest lesion there is a filling of the alveoli with polymorphonuclear leukocytes, alveolar phagocytes, occasional erythrocytes, and variable amounts of fibrin. There is similar exudate in some of the alveoli which surround the lesion, and the capillaries in the interstitial tissue are dilated and filled with blood. As the lesion progresses, caseation may occur or the defect may become organized by granulation and fibrous tissue into an oval or round tubercle. Until the introduction of streptomycin, recoveries from acute hematogenous tuberculosis were so infrequent that even clinical data on patients recovering from this disease were few. Recently, as a result of streptomycin treatment, numerous cases of recovery from

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[2] This investigation was supported (in part) by a research grant from the National Institutes of Health, Public Health Service; and (in part) by a grant from the Life Insurance Medical Research Fund.

[3] James Alexander Miller Research Fellow.

[4] The words "hematogenous" and "miliary" have been used throughout this paper to describe that form of tuberculosis which arises from the dissemination of tubercle bacilli by the blood stream. Although these terms are etymologically either incorrect or inexact when used in this sense, they are succinct and are commonly used to describe this disease.

this illness have been described (2, 3, 4, 5). Complete clearing of the abnormal shadows in the chest roentgenograms has been observed in many of these patients. In none of them have symptoms of pulmonary insufficiency been reported. Isolated pathologic studies in patients recovering from pulmonary hematogenous tuberculosis treated with streptomycin, but dying from other causes, have shown that the lesions in the lungs consist of small, fibrotic, well-localized, interstitial collections of collagen, with small numbers of lymphocytes, macrophages, and fibrocytes. Frequently, no tubercle bacilli are identified and no caseous material is present (6, 7).

Even with streptomycin, acute hematogenous tuberculosis frequently has a complicated and stormy course. This has prevented serial cardiopulmonary function studies in any large group of patients. Nevertheless, some information about the physiologic characteristics and course of this disease has been obtained, not only from repeated observations on a few patients but also from single observations on different patients in various stages of the disease.

Methods

The measurements of lung volume, maximum breathing capacity, ventilation, arterial oxygen saturation, and arterial carbon dioxide tension have been made by the methods recently described by Baldwin, Cournand, and Richards (8).

The oxygen tension difference between alveolar air and arterial blood (A-A gradient) while each of two different concentrations of oxygen is inspired (21 per cent and 16 per cent, as a rule) has been determined by the methods of Riley, Lilienthal, and others (9, 10). An analysis of these A-A gradients, which requires, in addition, a knowledge of the oxygen concentration of mixed venous blood, was carried out according to the theoretical considerations and methods described by Riley and others (11, 12, 13). According to these considerations, the A-A gradient may be divided into two components, namely, that which results from incomplete equilibrium between the oxygen tension of alveolar air and of capillary blood leaving the alveoli, and that which results from the admixture of mixed venous blood and pulmonary capillary blood. The analysis permits an estimation of the magnitude of each of these two components from which may be calculated the oxygen diffusing capacity of the lungs and the percentage of venous admixture in arterial blood. The *oxygen diffusing capacity* of the lungs is a measure of the permeability to oxygen of the alveolar-capillary membrane for the lung as a whole, and is dependent, not only on the permeability per unit area, but also on the size of the total area. It is expressed in cc. per minute per mm. of mercury of alveolar-capillary mean oxygen tension difference and normally has a value of greater than 15 at rest. The *venous admixture in the arterial blood* is made up of an actual volume of mixed venous blood, which reaches the peripheral arterial blood by anatomically distinct right to left shunts (bronchial veins, thebesian veins, et cetera), and a virtual volume of mixed venous blood, the calculation of which takes into account all the contributions of capillary blood from alveoli where ventilation is variably reduced in relation to perfusion. This total volume of mixed venous blood is expressed as a percentage of total pulmonary blood flow (cardiac output) and normally has a value of less than 6 per cent. Some quantitative estimation by this method may be made even in those instances in which mixed venous blood has not been obtained. By assuming pulmonary capillary-venous oxygen differences that are known to cover the likely physiologic range, one may determine the limits within which the diffusing capacity and the percentage venous admixture must lie. Finally, these methods also permit the calculation of the *dead space ventilation*. The calculation is based upon the relationship between the carbon dioxide

tension of alveolar air and that of expired air and upon the assumption that arterial and alveolar carbon dioxide tensions are equal (14). The dead space ventilation represents that portion of the total ventilation which takes no part in gas exchange. It is made up, not only of the actual volume of gas contained in the anatomic dead space, but also of a virtual volume of gas arising from the contribution of all alveoli where ventilation is maintained but circulation is reduced or absent. The dead space ventilation is expressed as a percentage of total ventilation, and its value normally does not exceed 30 per cent.

It is important to assess the magnitude of the possible errors which exist in the A-A gradient when it is measured by this method and to estimate how these errors would affect the final differentiation of defects in pulmonary gas exchange into the two components enumerated above. The errors involved in the calculation of the A-A gradient arise from two sources, namely, errors in the quantitative analysis of blood and gas, and errors arising out of minor deviations from the "steady state" during a study. Data are not available to determine precisely what deviations in the respiratory quotient (R.Q.), arterial carbon dioxide tension, and arterial oxygen tension can be ascribed to these two sources. However, reference to descriptions of the analytic methods employed (15, 16, 17) and observations in this laboratory indicate that assumed deviations of ± 0.03 for the R.Q., ± 2 mm. of mercury for the arterial carbon dioxide tension, and ± 4 mm. of mercury for the arterial oxygen tension are reasonable and probably not maximal. From these deviations, it can be shown that the A-A gradient may vary by ± 7 mm. of mercury. The effect of possible errors of this magnitude upon the results obtained from the further analysis of the A-A gradient by the method of Riley, Donald, and Cournand (13) is dependent upon the size of the gradients at the two levels. When the gradients are large, the final results, i.e., oxygen diffusing capacity and percentage venous admixture, are altered quantitatively by such errors but not qualitatively; but, when the gradients are small, the further analysis is either not possible or the results may be qualitatively incorrect. Because the frequency of occurrence of errors as large as ± 7 mm. of mercury cannot be determined, the effect of smaller deviations (e.g., ± 3 mm. of mercury) upon the final results has been examined. This examination shows that in cases with A-A gradients in the lower ranges, i.e., below 15 mm. of mercury, this small variation may alter qualitatively as well as quantitatively the final analysis of the A-A gradient.

Results

The data presented (tables 1–6) are based upon the study of 11 cases of acute hematogenous pulmonary tuberculosis. Studies were carried out at various intervals before, during, and up to three years after the start of streptomycin therapy. In addition, autopsy findings are available for 2 cases.

I. Physiologic observations

Acute stage: The observations indicate that the major physiologic derangements present in the early exudative phase were as follows:

1) All lung volumes were reduced and there was no evidence of pulmonary emphysema.
2) The maximum breathing capacity was normal or somewhat reduced. In some cases this reduction may have been related to the patients' poor general condition.
3) Marked hyperventilation during rest, exercise, and recovery was always present.
4) There was an interference with the transfer of oxygen from the alveolus to the arterial blood, which seemed to arise principally from a reduction in the

TABLE 1

Physical Characteristics and Clinical Diagnosis of 11 Cases of Hematogenous Pulmonary Tuberculosis

CASE	INITIALS	AGE	SEX	HEIGHT	WEIGHT	D.S. AREA	ADDITIONAL DIAGNOSES
				cm.	*kg.*	*sq.m.*	
1	E. B.	54	M	183	62	1.81	Pleurisy with effusion
2	J. Ru.	23	M	170	55	1.63	
3	R. M.	31	F	165	45	1.47	Tuberculous meningitis
4	J. Ri.	40	M	163	42	1.41	Pleurisy with effusion, tuberculous meningitis
5	R. G.	50	F	157	39	1.32	Bilateral pleurisy with effusion
6	E. E.	26	F	157	42	1.37	Tuberculous meningitis
7	E. M.	28	M	180	91	2.11	Tuberculous arthritis, right hip
8	F. A.	48	M	164	52	1.55	Chronic pulmonary tuberculosis, minimal; tuberculous arthritis, right knee
9	K. C.	31	F	164	67	1.73	Tuberculous meningitis, tuberculous spondylitis
10	F. R.	42	M	168	65	1.74	Tuberculosis of sternum, tuberculous epididymitis
11	J. M.	32	F	155	48	1.48	Tuberculous salpingitis, bilateral; tuberculous peritonitis

TABLE 2

Lung Volumes and Maximum Breathing Capacity in 11 Cases of Hematogenous Pulmonary Tuberculosis

CASE	TIME AFTER START OF STREPTOMYCIN TREATMENT	LUNG VOLUMES PER CENT OF PREDICTED VALUES			RESIDUAL VOLUME/TOTAL CAPACITY × 100	MAXIMUM BREATHING CAPACITY PER CENT OF PREDICTED VALUES
		Vital Capacity	Residual Volume	Total Capacity		
Normal		100	100	100	<35	100
1	4 days	55	100	67	36	106
2	5 days	56	66	76	17	67*
	6 weeks	74	63	70	17	106
3	10 weeks	79	100	85	31	98*
4	1 week	41	110	58	46	43*
	13 weeks	58	165	84	48	41*
	27 weeks	74	90	78	28	58*
	9 months	83	86	86	24	58*
5	3 weeks	38	80	53	39	63*
6	5 weeks	79	83	78	27	117*
7	3 months	54	52	53	20	87*
	9 months	60	44	57	15	119*
8	2 months	71	120	88	34	100*
	8 months	83	100	90	30	100*
9	19 months	64	69	67	27	94
10	12 months	99	79	101	19	135
	24 months	110	56	100	14	143
11	19 months	63	94	76	32	92
	32 months	67	83	74	29	96
	38 months	67	128	83	40	85

* Maximum breathing capacity in the supine position.

oxygen diffusing capacity of the lung. This is the most important and characteristic finding in this stage of the disease.

Late stage: Early in the course of treatment this marked interference with oxygen diffusion improved and milder physiologic defects remained.

1) A reduction in all lung volumes persisted. Evidence of pulmonary emphysema was present in only one case.

TABLE 3

Ventilation During Rest, Exercise, and Recovery; and Index of Intrapulmonary Mixing in 11 Cases of Hematogenous Pulmonary Tuberculosis

CASE	TIME AFTER START OF STREPTOMYCIN TREATMENT	VENTILATION LIT/MIN/SQ.M.B.S.			INDEX OF INTRAPULMONARY MIXING
		Basal	1 Minute Standard Exercise	1st Minute Recovery	
Normal		3.4	10.6	12.4	<2.5
1	4 days	5.7	16.9	20.7	1.2
2	5 days	6.7	—	—	1.4
	6 weeks	4.8	—	—	1.1
3	2 days before treatment	7.7	—	—	—
	10 weeks	4.1	—	—	1.0
4	1 week	9.8	—	—	1.0
	13 weeks	4.8	—	—	1.5
	27 weeks	4.2	—	—	0.9
	9 months	5.2	12.3	9.9	1.3
5	3 weeks	5.7	—	—	0.8
6	5 weeks	5.4	—	—	0.9
	6 months	3.3	—	—	—
7	3 months	3.9	—	—	1.1
	9 months	3.6	—	—	1.3
8	2 months	6.8	—	—	1.9
	4 months	7.6	—	—	—
	8 months	5.8	—	—	—
9	19 months	3.3	9.4	10.7	1.1
10	12 months	5.3	13.9	15.0	2.4
	24 months	4.3	12.8	14.7	1.5
11	19 months	4.8	16.6	15.6	1.7
	32 months	4.4	11.3	13.8	1.4
	38 months	5.6	—	—	1.2

2) The maximum breathing capacity was normal or only slightly reduced.
3) Hyperventilation during rest, exercise, and recovery was sometimes present but was always mild.
4) Reduction in the oxygen diffusing capacity was sometimes observed. The A-A gradients in this late stage, however, were always smaller than those which were observed in the early acute phase.
5) The physiologic dead space was elevated.
6) Pulmonary arterial hypertension during mild exercise was observed in one case.

One or more of these functional abnormalities were present in all patients, even when physiologic studies were carried out as late as three years after the start of treatment.

TABLE 4

Measurements of Gas Exchange in 11 Cases of Hematogenous Pulmonary Tuberculosis

CASE	TIME AFTER START OF STREPTOMYCIN TREATMENT	OXYGEN INTAKE CC/MIN/SQ.M.B.S.		OXYGEN INTAKE IN CC/LIT. OF VENTILATION		ARTERIAL BLOOD		
						Oxygen Saturation		pCO_2 REST MM. OF MERCURY
		Rest	1 Minute Standard Exercise	Rest	1 Minute Standard Exercise	Rest	1st Minute Recovery	
						per cent	*per cent*	
Normal		132	494	45.1	55.8	98	98	39
1	4 days	142	439	30.8	32.0	97	89	—
2	5 days	170	—	30.4	—	94	—	30
	6 weeks	136	—	34.6	—	94	—	41
3	2 days before treatment	182	—	28.8	—	91	—	28
	10 weeks	86	—	26.2	—	95	—	29
4	1 week	162	—	20.1	—	91	—	43
	13 weeks	115	—	25.8	—	96	—	40
	27 weeks	111	—	28.4	—	98	—	40
	9 months	103	410	24.0	40.1	94	92	31
5	3 weeks	155	—	32.6	—	97	—	36
6	5 weeks	132	—	29.0	—	97	—	38
	6 months	93	—	33.7	—	99	—	45
7	3 months	130	—	40.9	—	95	—	36
	9 months	135	—	45.0	—	96	—	39
8	2 months	142	—	25.7	—	94	—	34
	4 months	147	—	23.5	—	93	—	34
	8 months	141	—	29.5	—	93	—	34
9	19 months	109	429	35.8	55.5	97	95	43
10	12 months	139	523	31.4	45.5	95	98	31
	24 months	127	553	35.1	51.9	98	98	40
11	19 months	136	418	33.8	30.2	94	93	38
	32 months	119	313	32.5	33.3	95	94	42
	38 months	111	—	24.0	—	92	—	40

II. ILLUSTRATIVE CASES

Case Illustrating Early, Exudative Phase of Acute Hematogenous Tuberculosis

Case 1

Clinical history: Fourteen months before admission to Bellevue Hospital, this 54-year-old Negro male developed abdominal symptoms and fever. He was studied in another hospital and a cause for these complaints was not found. A roentgenogram of the chest taken at this time was said to have been normal. The symptoms continued and, seven months before admission, evidence of a right-sided pleural effusion was found by roentgenography; shortly thereafter tuberculosis of a cervical lymph node was diagnosed by examination of biopsy material. He was transferred to a sanatorium. Tubercle bacilli

were found by gastric culture, the pleural fluid persisted but did not increase, and low-grade fever continued.

TABLE 5

Measurements of Ventilation-Perfusion Relationships and Oxygen Diffusing Capacity of the Lung in 11 Cases of Hematogenous Pulmonary Tuberculosis

CASE	TIME AFTER START OF STREPTOMYCIN TREATMENT	DEAD SPACE / TIDAL VOLUME × 100	EFFECTIVE ALVEOLAR VENTILATION LIT/MIN/SQ.M.B.S.	ALVEOLAR OXYGEN TENSION WHILE BREATHING ROOM AIR	A-A GRADIENT WHILE BREATHING ROOM AIR	A-A GRADIENT WHILE BREATHING 16 PER CENT OXYGEN	$D_{L_{O_2}}$*	VENOUS ADMIXTURE / CARDIAC OUTPUT × 100
Normal		<30	2.3	100	<12	<12	>15	<6
1	4 days	38	3.5	107	23	21	(6 to 9)	(5 to 15)
2	5 days	30	4.7	111	45	36†	(6 to 10)	(14 to 45)
	6 weeks	37	3.0	98	11	3	>21	5
3	2 days before treatment	32	4.9	112	44	30†	‡	‡
	10 weeks	27	3.0	114	35	19	‡	‡
4	1 week	60	4.7	99	24	41	(4 to 5)	(1 to 5)
	12 weeks	41	3.8	103	5	6	—	—
	13 weeks	39	3.2	108	9	4	>15	3
	27 weeks	33	2.8	105	21	15	7	7
	9 months	40	3.1	113	19	14	6	5
5	3 weeks	37	3.6	110	21	11	(7 to 12)	(4 to 15)
6	5 weeks	26	4.0	110	14	10	(6 to 11)	(3 to 10)
	6 months	33	2.7	104	5	0	>13	2
7	3 months	21	3.1	106	20	11	(12 to 25)	(5 to 16)
	9 months	39	2.2	103	19	10	(12 to 23)	(5 to 16)
8	2 months	44	3.8	106	26	17	(7 to 10)	(7 to 22)
	4 months	44	4.2	108	25	13	11	9
	8 months	35	3.8	105	27	17	(6 to 10)	(7 to 25)
9	19 months	29	2.7	106	10	6	12	4
10	12 months	30	3.7	116	27	12	15	13
	24 months	33	2.8	103	9	−1	(>13)	(2 to 7)
11	19 months	43	2.7	103	27	17§	(10 to 13)	(7½ to 28)
	32 months	36	2.8	104	11	4§	(13 to 23)	(3 to 7)
	38 months	40	3.4	111	23	—	—	—

* Oxygen diffusing capacity of the lungs.
† Inspired oxygen concentration = 17 per cent.
‡ Arterial pO_2, while breathing low oxygen not low enough to permit analysis.
§ Inspired oxygen concentration = 12 per cent.
() Estimated from assumed capillary-venous differences of 15 per cent and 50 per cent.

He entered Bellevue Hospital on January 26, 1950. His temperature was 99.8°F., pulse 88, respiration 16 per minute, and blood pressure was 120 systolic and 84 diastolic in mm. of mercury. Signs of a small right-sided pleural effusion were present. A roentgenogram of the chest (figure 1) still showed the pleural effusion, and for the first time evidence of miliary pulmonary tuberculosis was visible, with fine nodular shadows uniformly scattered throughout both lung fields.

Streptomycin, 2 gm. daily, and para-aminosalicylic acid (PAS), 12 gm. daily, were started on February 16.

Pulmonary function studies: Pulmonary function studies, done four days after the start of streptomycin treatment, showed:

a) A decrease in the vital capacity and total capacity, with a normal residual volume and a normal index of intrapulmonary mixing
b) A normal maximum breathing capacity
c) Hyperventilation during rest, exercise, and recovery

TABLE 6

Hemodynamic Measurements in 7 Cases of Hematogenous Pulmonary Tuberculosis

CASE	TIME AFTER START OF STREPTOMYCIN TREATMENT	PULSE RATE	OXYGEN CONSUMPTION	A-V OXYGEN DIFFERENCE	CARDIAC INDEX	BLOOD PRESSURE, MM. OF MERCURY			
						Pulmonary Artery		Systemic Artery	
						S/D	Mean	S/D	Mean
			cc/min/ sq.m.B.S.	*cc/liter*	*lit/min/ sq.m.B.S.*				
Normal			126	30	3.27	20/9	13	112/65	84
			(406)	(73)	(5.40)	(13/4)	(11)	(125/78)	(100)
2	6 weeks	97	136	34	4.01	15/7	11	126/74	93
4	13 weeks	85	112	42	2.65	12/3	8	104/71	84
	27 weeks	65	117	49	2.38	23/6	14	114/71	92
	9 months	62	115	50	2.32	25/8	15	125/69	97
		(73)	(155)	(56)	(2.58)	(32/12)	(23)	(148/82)	(110)
6	6 months	60	79	33	2.39	10/3	6	137/95	114
		(—)	(—)	(—)	(—)	(16/9)	(12)	(156/104)	(129)
8	4 months	80	147	48	3.04	20/7	13	108/71	88
		(88)	(251)	(67)	(3.74)	(24/7)	(14)	(131/79)	(100)
9	19 months	60	112	45	2.49	23/7	13	128/85	104
		(94)	(288)	(61)	(4.72)	(19/4)	(12)	(125/78)	(98)
10	12 months	75	135	38	3.55	15/5	11	112/67	84
		(100)	(348)	(66)	(5.28)	(21/7)	(14)	(140/83)	(108)
11	38 months	80	111	35	3.16	19/7	13	—	—

( ) = measurements made during steady state exercise.

d) A normal arterial oxygen saturation at rest but a fall in the arterial oxygen saturation to 89 per cent after exercise
e) Increased alveolar-arterial oxygen tension gradients while breathing room air and 16 per cent oxygen
f) An increase in the physiologic dead space.

Course: Streptomycin and PAS were continued. The temperature returned to normal within two days. The miliary lesions in the lungs persisted. There was a decrease in the pleural effusion. On April 18 he left the hospital against advice.

Comment: This patient is of particular interest because it was possible to conduct physiologic studies early in the course of pulmonary hematogenous tuberculosis, while the patient was in good physical condition and without fever. The presence of a pleural effusion slightly complicated the interpretation of the results. The reduction in the vital capacity and total capacity can be partially

attributed to the presence of this hydrothorax. The arterial oxygen unsaturation after exercise, the increased alveolar arterial oxygen tension gradient while breathing 16 per cent oxygen, and the increased physiologic dead space cannot, in the writers' experience, be accounted for by a pleural effusion of such small extent and should be attributed to the hematogenous pulmonary tuberculous lesions. The alveolar-arterial gradients of 23 mm. of mercury while breathing room air, and of 21 mm. while breathing 16 per cent oxygen establish, within the limits of the methods, the fact that there is an interference with the diffusion of oxygen across the alveolar capillary membrane. Because mixed venous blood

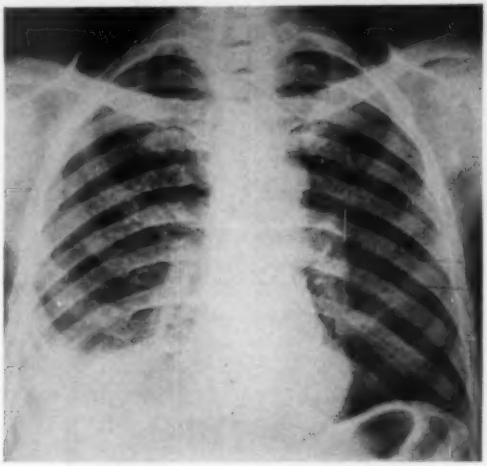

FIG. 1. Case 1. Before streptomycin therapy. A small right pleural effusion is present and fine nodular infiltrations are distributed throughout both lung fields.

was not obtained during this study, the diffusing capacity cannot be calculated accurately by the graphic method which Riley, Donald, and Cournand (13) have described. However, by assuming capillary-venous oxygen saturation differences throughout the likely physiologic range of 15 per cent to 50 per cent, it can be shown that the diffusing capacity as calculated by this method is reduced. The use of these same assumptions does not define whether the degree of physiologic venous admixture was normal or elevated.

Case Illustrating Physiologic Evolution of Extensive, Acute Miliary Tuberculosis During Nine Months of Treatment. Autopsy

Case 4

Clinical history: This 40-year-old Puerto Rican male was admitted to Bellevue Hospital on June 22, 1949, complaining of weight loss, malaise, and anorexia of three weeks' duration. Cough, productive of small amounts of non-foul, non-bloody sputum, developed three weeks before admission and was followed by pleuritic pain on the right side and high fever. He became short of breath and came to the hospital. He had lost an unknown amount of weight.

The patient was emaciated but in no acute distress. Temperature was 103.8°F., respiration 24, pulse 96 per minute, and blood pressure 112 systolic and 78 diastolic in mm. of mercury. There were signs of fluid at the right base.

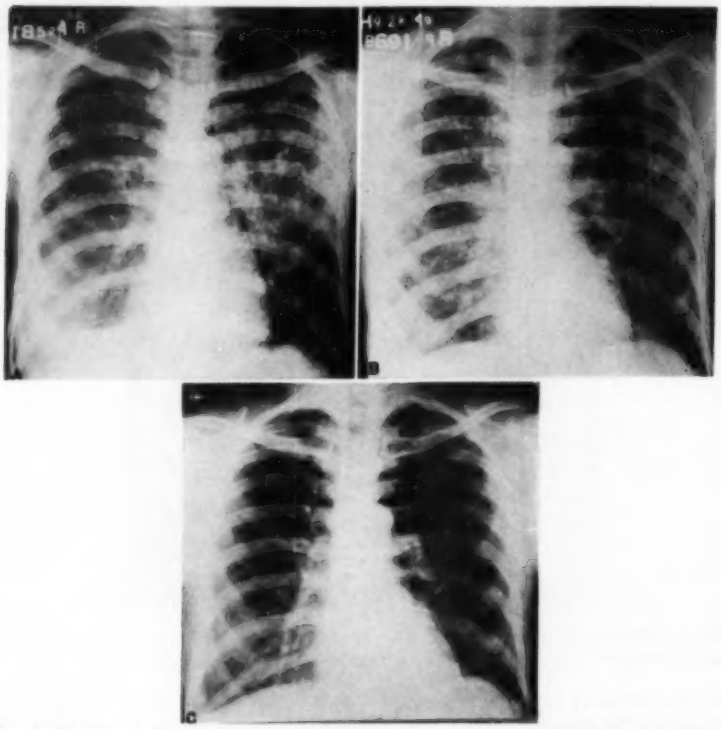

FIG. 2. Case 4. (A) Before streptomycin therapy. A small right pleural effusion is present and there are conglomerate infiltrations with indistinct borders throughout both lungs. (B) After three months of streptomycin therapy. There has been partial resolution of the pleural effusion and the pulmonary infiltration. (C) After nine months of streptomycin. Complete clearing has occurred.

A roentgenogram of the chest (figure 2A) showed a small right pleural effusion and nodular non-discrete densities with indistinct borders throughout both lung fields. The sputum was negative for acid-fast bacilli.

Course: A right thoracentesis yielded 250 cc. of fluid. Beginning on June 30, 2 gm. of dihydrostreptomycin and 12 gm. of PAS were administered daily. The dihydrostreptomycin was continued for 180 days; the PAS, throughout the patient's hospitalization. The nodular densities became more sharply defined, and the pleural effusion showed gradual clearing during the first month of treatment (figure 2B). In October, choroidal tubercles were noted, and signs of meningitis appeared. Intrathecal streptomycin was given for several weeks on alternate days.

The low-grade fever disappeared in the sixth month of hospitalization, and by the ninth month the chest roentgenogram (figure 2C) was normal except for blunting of the right costophrenic angle and a calcified lesion in the left lung field.

In July 1950, signs of tuberculous meningitis returned. Intrathecal and intramuscular streptomycin were resumed but his condition continued to deteriorate, and death occurred on September 1, 1950.

Pulmonary function studies: Pulmonary function studies were done on this patient at various stages of treatment and showed the following significant findings (tables 2–6):

a) Early in treatment, there was a reduction in the vital capacity and total capacity, with an abnormally high residual volume. Later during treatment, the vital capacity and the total capacity increased and the residual volume returned to normal predicted values. The index of intrapulmonary mixing was always normal.

b) The maximum breathing capacity was always low, but increased during treatment.

c) Hyperventilation was always present, but decreased during treatment.

d) The arterial oxygen saturation at rest was slightly reduced at the earliest study but became normal during treatment. Arterial oxygen unsaturation was observed after exercise late in the period of treatment.

e) The degree of physiologic venous admixture was always normal, indicating that the pulmonary blood flow was distributed through well-ventilated areas, whereas the persistently high physiologic dead space suggested ventilation of non-perfused alveoli.

f) There was a reduction in the oxygen diffusing capacity in the first week, which returned to normal twelve weeks after the start of treatment. At twenty-seven and thirty-six weeks, it was again reduced.

g) The pulmonary artery pressures were normal at rest and were associated with a low cardiac output during the entire period of observation.

h) At the final study, on mild exercise, a significant increase in the pulmonary arterial pressure occurred with only a slight increase in cardiac output.

Autopsy: The significant autopsy findings were limited to the lung and meninges. Obliteration of the pleural space by fibrous adhesions, a calcified focus in the left lung, a fibrocaseous nodule at the apex of the right lung, and innumerable small areas of fibrosis throughout both lungs could be identified in the gross specimen. Microscopically, the lung (figure 3) showed numerous fibrous areas. These were associated with, and adjacent to, the smaller branches of the pulmonary artery, and in places these nodules could be identified in the alveolar-capillary septa. Focal emphysema was adjacent to some of the areas of scarring. Tubercles could be identified only in the small apical fibrocaseous nodule. The meninges showed the characteristic changes of tuberculous meningitis.

Comment: This patient had the most extensive pulmonary involvement of any patient in this series both symptomatically and from roentgenographic appearance. At the end of nine months, despite the normal appearance of the roentgenogram, the low oxygen diffusing capacity and the elevation of the pul-

monary arterial pressure following a very small increase in cardiac output suggest that the pulmonary vascular bed was markedly reduced and that pathologic residues were present. The pathologic findings seemed to verify these conclusions.

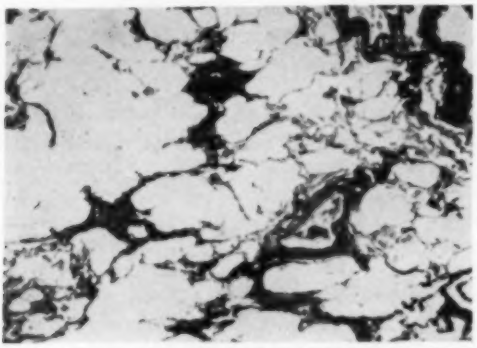

FIG. 3. Case 4. Photomicrograph of the lung. In the alveolar capillary septa and about some of the small vessels there are areas of fibrosis.

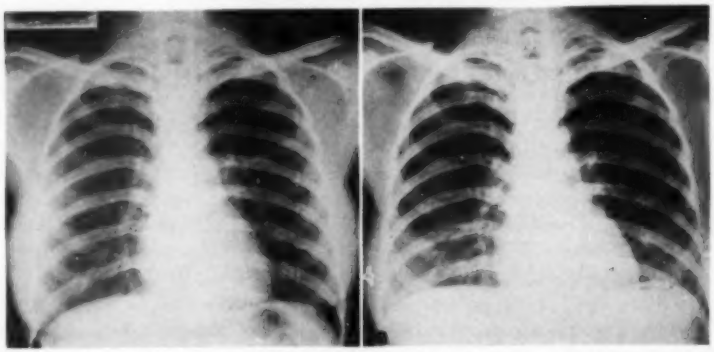

FIG. 4. Case 6. (A) Before streptomycin therapy. There is a very fine, discrete, nodular infiltration throughout both lungs. (B) After four weeks of streptomycin therapy. Nearly complete resolution of the nodular infiltration has occurred.

Case Illustrating Clearing of Less Extensive, Acute Miliary Tuberculosis. Autopsy

Case 6

Clinical history: A 26-year-old Negro female was referred to Bellevue Hospital on December 26, 1949 with a diagnosis of tuberculous meningitis. Approximately three and one-half weeks before admission, headache and fever had developed, followed by nausea and vomiting and, finally, stiffness of the neck. She was admitted to another hospital where, in addition to tuberculous meningitis, the characteristic stippling of miliary tuberculosis was noted in the chest roentgenogram (figure 4A).

Course: Intramuscular and intrathecal streptomycin were started with marked improvement in the meningitis and clearing of the chest roentgenogram. On admission to Bellevue her symptoms were fever, weakness, and slight dizziness. Sputum was negative for acid-fast bacilli. A roentgenogram of the chest showed only fine reticular strands in the right lower lung field (figure 4B).

Lung function studies: Lung function studies done January 17, approximately five weeks after the beginning of streptomycin treatment, were normal except for a slight reduction in the vital capacity and total capacity and a small degree of hyperventilation.

Subsequent course: The pulmonary lesions had completely disappeared on roentgenogram by February 16, 1950. Stiffness of the neck and evidence of active tuberculous meningitis returned.

Cardiopulmonary studies: Cardiopulmonary studies, including cardiac catheterization, two level oxygen tension studies, and "steady state" exercise were done May 25, 1950. They showed the following significant findings (table 4):

a) A low oxygen intake and a low cardiac index
b) Normal alveolar arterial oxygen tension gradients, with normal diffusing capacity and no abnormal venous admixture
c) No pulmonary artery hypertension with mild exercise.

Autopsy: In spite of intrathecal streptomycin therapy, the patient's condition grew worse, and she died on July 30, 1950. The significant autopsy findings were limited to the lungs and meninges. A few fibrous pleural adhesions were present but no other lesion could be recognized grossly in the lungs. On microscopic examination, small areas of interstitial and septal thickening were found. This thickening was formed by fibrous proliferation and was infiltrated by lymphocytes and mononuclear cells. No thickening of the vessel walls was noted although some of the scars were perivascular. The lesions seemed less extensive than in Case 4. The meninges showed the characteristic changes of tuberculous meningitis.

Comment: These studies indicate that, in this patient after complete disappearance of the miliary lung lesions from the chest roentgenogram, pulmonary gas exchange was normal. Unlike the previous case in which the original disease was more extensive, the pulmonary vascular bed in this patient was not sufficiently reduced to cause hypertension in the lesser circulation at rest or during mild exercise. During the second study the meningitis appeared to be progressing, and the patient was emaciated. The low oxygen intake and the low cardiac index at this time may well reflect the cachectic state associated with meningitis.

Case Illustrating Healed Miliary Tuberculosis

Case 10

Clinical history: A 42-year-old Puerto Rican male was admitted to the Psychiatric Division of Bellevue Hospital on February 2, 1947 with a four-month history of slight cough, malaise, and diarrhea. The physical examination showed a disoriented patient with a temperature of 104.2°F. and a liver palpable 8 cm. below the right costal margin. Fever continued, slight jaundice developed, and soft diffuse lesions characteristic of miliary

tuberculosis were discovered by roentgenogram to be present in the lung fields (figure 5A). Miliary tubercles were noted on ophthalmoscopic examination.

Course: On March 7, 1947 administration of streptomycin, 3 gm. daily, was commenced and was continued for 60 days. The sensorium improved; the temperature returned to normal, and a chest roentgenogram showed gradual but complete clearing of the lung fields. On October 30, a left epididymectomy was performed for a nodular lesion which, on pathologic examination, was found to be tuberculous. One sputum sample was found to be positive for acid-fast bacilli. The patient improved following operation and was well three years after the beginning of streptomycin treatment.

Physiologic studies: Physiologic studies were performed one and two years after the beginning of streptomycin treatment, when the chest roentgenogram

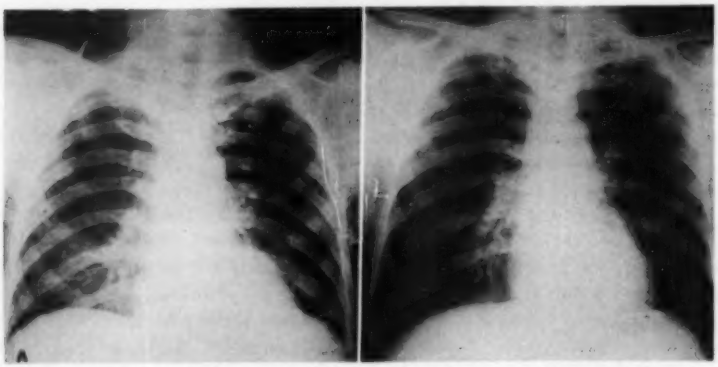

FIG. 5. Case 10. (A) Before streptomycin therapy. There are irregular, fine nodular, infiltrations throughout both lung fields. (B) After one year of streptomycin therapy. Complete resolution of the infiltration has occurred.

(Figure 5B) appeared normal. Except for slight hyperventilation during rest, exercise, and recovery and an increase in the physiologic venous admixture, the findings were all within normal limits.

Comment: Two years after the beginning of streptomycin treatment, the only abnormality in pulmonary function in this patient with hematogenous pulmonary tuberculosis was slight hyperventilation and evidence of some perfusion of areas where gas exchange did not take place. The normal pulmonary arterial pressure during mild exercise indicates that in this case the pulmonary vascular bed has not been markedly reduced.

DISCUSSION

The observations reported here indicate that hematogenous tuberculosis of the lungs causes distinct pulmonary functional abnormalities in both the acute inflammatory phase and after streptomycin therapy has produced clinical recovery. The present physiologic observations may be correlated, not only with the pathologic observations which have previously been made in various stages

of this disease, but also with the physiologic patterns which have been observed in other groups of pulmonary disease.

The physiopathologic characteristics of widespread hematogenous pulmonary tuberculosis in the acute, exudative phase are identical to those which have recently been described in the syndrome of "alveolar-capillary block" (18). The major physiologic abnormality in this syndrome was found to be an impairment to the diffusion of oxygen across the aveolar-capillary membrane. The pathologic finding of widespread thickening of the alveolar capillary septa by fibrous or granulomatous infiltration was thought to account for the major physiologic observation. In the early stage of miliary tuberculosis when a similar impairment of oxygen diffusion exists, it is probable that inflammatory involvement of this septal area is present. In addition, miliary tubercles beginning in or around pulmonary capillaries would obliterate portions of the capillary bed, reduce the total area available for gaseous diffusion, and thus act to further impair oxygen diffusion. The finding of normal, or only slightly reduced, arterial oxygen saturation while breathing room air at rest is not incompatible with the finding of reduced oxygen diffusing capacities. In spite of a reduced oxygen diffusing capacity, if alveolar ventilation is normal, the pulmonary capillary blood may be almost fully saturated when one breathes ambient air at sea level. This occurs because of the shape of the oxyhemoglobin dissociation curve in its upper portion.

In the previously described cases with "alveolar-capillary block," a marked increase in the percentage of venous admixture was present only in the more advanced cases. It was believed that this increase in venous admixture resulted from the perfusion of areas where the fibrous or granulomatous process had become so far advanced as to produce true venous shunts. In hematogenous tuberculosis, however, it seems clear from the pathologic description of alveolar exudate in the acute phase that the increased venous admixture may arise from the perfusion of alveoli where ventilation is either absent or reduced.

With effective treatment, these abnormalities of oxygen transfer return toward normal. Of particular interest is the observation that interference with the diffusion of oxygen may disappear as early as three weeks after the start of streptomycin therapy. This correlates well with the pathologic finding that the earliest change which occurs in streptomycin-treated tuberculosis is a resorption of the acute inflammatory exudate (7).

Some of the remaining physiologic defects of this early phase can be correlated with pathologic findings. The reduced lung volumes must be caused by an encroachment of the interstitial and intra-alveolar exudate upon the breathing space. The marked hyperventilation occurs in the absence of significant arterial anoxia and in the presence of a normal or reduced arterial carbon dioxide tension. The inflammatory process in the lungs could give rise to ventilatory stimulation via the pulmonary stretch reflexes, and initiate hyperventilation. Once established, this hyperventilation would cause a lowering of the arterial carbon dioxide tension and a lowering of the alkaline reserve. Studies at high altitude have shown (19) that such a decrease in the alkaline reserve will in-

crease the sensitivity of the respiratory center for carbon dixode. Finally, the elevated physiologic dead space observed in this early phase probably results from the persistent ventilation of areas where the inflammatory process has interfered with perfusion.

In spite of clinical recovery and in some instances of complete roentgenographic clearing, pulmonary dysfunction has been found to remain as long as thirty-eight months after the beginning of streptomycin therapy. With few exceptions, the derangements are identical with those which have been previously described in the pulmonary fibroses associated with mild ventilatory insufficiency (20, 21). The innumerable small, interstitial scars which remain in the healed stage of miliary tuberculosis cause the reduction in lung volumes, mild hyperventilation, and minor defects in ventilation-perfusion relationships which are characteristic of this type of pulmonary insufficiency. In one case, an absolute increase in the residual volume and an increase in the residual volume to total capacity ratio were observed. This suggests that significant emphysema may be a late complication of this disease as it is in some of the other pulmonary fibroses. A reduced oxygen diffusing capacity was found in some cases long after streptomycin treatment was begun. The reduction of the pulmonary vascular bed which must accompany the healing and scar formation which is observed late in this disease could result in some reduction of the diffusing capacity of the lung.

That the fibrous residues of healed miliary tuberculosis may in some cases result in obliteration of a large portion of the pulmonary vascular bed is demonstrated by the finding of pulmonary artery hypertension on mild exercise in one case. Studies which have previously been reported in normal persons (22) and in patients after pneumonectomy (23) indicate that such an increase in pulmonary arterial pressure occurs only with a marked decrease in the pulmonary vascular bed. One can only speculate at the present time on whether this obliteration of the pulmonary vascular bed, with its accompanying mild pulmonary arterial hypertension on exercise, will eventually lead to right ventricular hypertrophy and congestive failure. These complications have as yet not been reported in cases of healed hematogenous pulmonary tuberculosis.

Summary

The results of cardiopulmonary function studies in 11 patients with hematogenous pulmonary tuberculosis have been presented. These studies were carried out before and at various times during streptomycin treatment and after clinical recovery had taken place.

The observations indicate that (a) in the early phase, the pattern of pulmonary dysfunction is identical to that which has been reported in the syndrome of "alveolar-capillary block." The principal defect is an interference with oxygen transfer from the alveolous to the arterial blood, which arises chiefly from a reduction in the oxygen-diffusing capacity of the lung. (b) In the late phase, after clinical recovery has occurred, the pattern is like that which has been described in pulmonary fibrosis with mild ventilatory insufficiency.

The finding of pulmonary artery hypertension after mild exercise in the phase of clinical recovery suggests that this disease may produce a large reduction in the pulmonary vascular bed.

Sumario

La Función Cardiopulmonar en la Tuberculosis Pulmonar Hematógena Antes y Después de la Estreptomicinoterapia

Los resultados presentados representan estudios de la función cardiopulmonar en 11 enfermos con tuberculosis pulmonar hematógena. Esos estudios fueron llevados a cabo antes de la estreptomicinoterapia, en varias ocasiones durante la misma y después de tener lugar la reposición clínica.

Las observaciones realizadas indican que (a) en la fase temprana, el patrón de la disfunción pulmonar es idéntico al comunicado en el síndrome del "bloqueo alvéolocapilar." La principal deficiencia consiste en una obstrucción del pase de oxígeno del alvéolo a la sangre arterial, debido mayormente a reducción en la capacidad del pulmón para difundir oxígeno. (b) En la fase tardía, después de ocurrir la reposición clínica, el patrón es parecido al ya descrito en la fibrosis pulmonar con leve insuficiencia ventiladora.

El hallazgo de hipertensión en la arteria pulmonar después de un ejercicio ligero en la fase de la reposición clínica sugiere que la enfermedad en cuestión puede producir una gran reducción del lecho vascular en el pulmón.

Resume

La fonction cardiopulmonaire dans la tuberculose pulmonaire hématogène, avant et après la streptomycinothérapie

Les résultats de l'étude de la fonction cardiopulmonaire chez 11 patients atteints de tuberculose pulmonaire hématogène sont présentés. Ces études ont été entreprises à différents moments, avant et au cours du traitement par la streptomycine, et après que la guérison clinique ait pris place.

Les observations indiquent que (a) dans la phase de début le type de dysfonction pulmonaire est identique à celui qui a été décrit dans le syndrome "du bloc alvéolocapillaire". Le principal défaut est une interférence dans le transport de l'oxygène de l'alvéole au sang artériel, qui est principalmente déterminée par une réduction de la capacité de diffusion de l'oxygène du poumon. (b) Dans la phase tardive, quand la guérison clinique s'est produite, ce trouble fonctionnel est celui qui a été décrit dans la fibrose pulmonaire avec insuffisance modérée de la ventilation.

La présence de l'hypertension de l'artère pulmonaire à la suite d'un exercice léger, au cours de la phase de guérison clinique, suggère que cette affection peut provoquer une réduction importante du réseau vasculaire du poumon.

Acknowledgment

The authors gratefully acknowledge their indebtedness to Dr. Marvin Kuschner, who prepared the photomicrograph presented here and discussed with the authors many of the pathologic aspects of hematogenous pulmonary tuberculosis as it appeared in these patients.

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EXTRACTION AND FRACTIONATION OF WATER-SOLUBLE COMPONENTS FROM TUBERCLE BACILLI[1,2,3]

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INTRODUCTION

Seibert (1) and Bevilacqua and McCarter (2), by fractionating unheated culture filtrates, have shown that a more homogeneous tuberculin could be prepared if the proteins were not denatured by heating. Seibert (1) has also shown that the tuberculin prepared in the usual manner from heated culture filtrates is less active than the tuberculin obtained from unheated culture filtrates. This suggested that possibly the extraction of cells directly, without the intervention of autolytic processes, might yield proteins in a more native state than those present in the culture filtrates. In a previous publication (3) it was shown that, if the cells were ruptured mechanically and extracted with a phosphate buffer containing ethyl ether, a more potent tuberculin might be obtained. Chargaff and Saidel (4) reported the extraction of nucleoprotein from avian tubercle bacilli by the use of a borate buffer. As a consequence, in the present study, borate, as well as a phosphate buffer, was investigated as a means of extracting proteins from tubercle bacilli with a minimum of denaturation.

Various substances, in addition to the protein with a high tuberculin activity, have been isolated from cell extracts but none has been obtained in a pure form. Some of the characteristics of the different fractions are described.

MATERIALS AND METHODS

Culture and medium: In some of the preliminary experiments a lot of 100 gm. of lyophilized tubercle bacilli[5] was used. All other experiments were based on fresh cells from two- to four-week-old cultures of a human strain of *Mycobacterium tuberculosis* (TB I)[6]. The organisms were grown at 37° to 38°C. in the liquid medium described by Dubos and Middlebrook (5) according to the cultural procedures which they described.

Harvesting of the cells: The procedure for harvesting the culture has been described previously (3). After the cells were collected in a Büchner funnel they were washed with either borate[7] or phosphate[8] buffer.

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[3] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

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[5] Supplied by Dr. F. Stimpert of Parke, Davis and Company, Detroit, Michigan.

[6] TB I is the number of a stock culture in the Department of Bacteriology, University of Wisconsin, Madison. The culture originally came from the Bureau of Animal Industry.

[7] The buffer contains 0.1 M borate and 0.05 M citrate at pH 8.4.

[8] The buffer is a mixture of equal volumes of 0.85 per cent NaCl and 0.1 M phosphate buffer at pH 7.3.

Rupturing and extraction of the cells: The apparatus and the techniques used for rupturing the cells are described in a previous publication (3), together with the details of the ether and phosphate buffer extraction. For the borate buffer extraction, approximately 150 ml. of buffer were added to 20 gm. of cell debris and these were thoroughly mixed by shaking. The mixture was allowed to stand at least one hour before centrifugation. The cell debris was extracted three times in this manner. The pooled extracts were sterilized by successive passage through a No. 10, a No. 02, and finally through a No. 03 Selas filter. The smaller lots were dialyzed against distilled water and lyophilized, but the larger volumes were concentrated and freed of salt by ultrafiltration, using the apparatus described by Heckly and Watson (6).

Physical methods: The wet weight of cells was obtained by weighing the tubes used for shaking before and after filling with the moist cell paste. For purposes of calculating the yields, an aliquot of the moist cell paste, taken before shaking, was dried in a desiccator to determine the dry weight of cells. Aliquots of the extracts were lyophilized to determine the total yield and to provide material for the various analyses.

The Tiselius (7) apparatus was employed to obtain the electrophoretic patterns. The concentration of the material at the time of electrophoretic examination varied because of technical difficulties imposed by the high viscosity of some samples. An Ostwald viscometer, having an efflux time of 68 seconds with distilled water at 25°C., was used to measure viscosity. The procedure followed is described by Kabat and Mayer (8).

Chemical analytical methods: Desoxyribonucleic acid was determined by the use of Dische's (9) diphenylamine reagent as described by Kabat and Mayer (8). Carbohydrate was estimated by the use of the anthrone reagent described by Morris (10), using a purified tuberculin polysaccharide as a primary standard. The samples for the nitrogen determination were digested with sulfuric acid and copper selenite catalyst. The determinations were made by micro-Kjeldahl or nesslerization procedures.

Biological activities: The tuberculin activities of the preparations were compared with PPD-S,[9] using guinea pigs sensitized with heat-killed *M. tuberculosis* and tuberculous guinea pigs. For testing, both the standard PPD and the unknown preparations were dissolved at 1 to 10 mg. per ml. in a sterile phosphate buffer[10] as recommended by Seibert (11). The stock solutions, ½ ml. per vial, were frozen in dry ice and kept at −30°C. until ready for use. Buffered saline[11] was used to dilute the stock solution before testing. One-tenth milliliter of varying concentrations of one or more of the test preparations was injected intracutaneously on the right side, and known amounts of the standard, PPD-S, were injected on the left side of the guinea pig. The two axes of the ellipsoidal reaction were measured with calipers at twenty-four and forty-eight hours, and the thickness of the lesion was estimated on a scale of 1 to 4.

The antigenicity of the various extracts was compared by injecting a group of normal guinea pigs at weekly intervals with 0.1 ml. of buffered saline containing 50 γ of the test material.

Fractionation: Except for a few minor changes, the method used for removing most of the nucleic acid was essentially the same as that described by Heckly and Watson (3). The crude extracts, after being concentrated and washed with distilled water by ultrafiltration, were diluted with an equal volume of 0.2 M phosphate buffer, pH 7.2. In a typical calcium phosphate adsorption, 100 ml. of a concentrated extract, containing 6 mg. solids per ml., were diluted with 100 ml. of 0.2 M phosphate buffer. Ten milliliters of 0.5 M $CaCl_2$ were added slowly, and 0.1 M NaOH was added to maintain the pH above 5.5. The precipitate was removed by centrifugation and washed twice with 0.01 M phosphate buffer at

[9] The PPD-S, which is the tuberculin standard in the United States, was kindly supplied by Dr. Florence Seibert, Henry Phipps Institute, University of Pennsylvania, Philadelphia.

[10] Solution which contains 4.774 gm. $Na_2HPO_4 \cdot 12\ H_2O$ and 0.363 gm. KH_2PO_4 per liter with 100 ml. of 5 per cent phenol added after sterilization.

[11] Solution which contains 4.774 gm. $Na_2HPO_4 \cdot 12\ H_2O$ and 0.363 gm. KH_2PO_4 and 8 gm. NaCl per liter. The pH was 7.3 after sterilization.

pH 6.5. Calcium chloride was added five times. The pooled precipitates then were dissolved in 0.5 M citrate buffer at pH 5.5 and the salts were removed either by dialysis or by ultrafiltration before lyophilization.

The supernatant solutions were concentrated and freed of salts by ultrafiltration before being lyophilized.

In one attempt to purify the crude nucleic acid preparation, obtained by adsorption of an ether-phosphate buffer extract, the solution was acidified with hydrochloric acid to pH 3.0. The precipitate which formed was removed by centrifugation and washed with distilled water. The precipitate was resuspended in distilled water and dissolved by adjusting the pH to 6.5 with sodium hydroxide. The supernatant solution was neutralized and dialyzed against distilled water before being lyophilized.

TABLE 1

A Schematic Diagram of an Ethanol Fractionation of a Borate Buffer Extract of Mycobacterium Tuberculosis var. Hominis

110 mg. of extract, free of nucleic acid, were dissolved in 6 ml. of 0.03 M KH_2PO_4. The pH was 6.3. Enough 0.5 M acetic acid was added to bring the pH to 5.3, with the final calculated ionic strength at 0.04 M. The solution became opalescent and a precipitate formed on cooling to 0°C.

Centrifuged

Precipitate I	Supernatant I
Washed with 1 volume of water, then with 2 volumes of 15 per cent ethanol.	Volume 10 ml., adjusted to 15 per cent ethanol with 95 per cent ethanol, and temperature lowered to −5°C. as the ethanol was added.
Precipitate dissolved in water and lyophilized.	Centrifuged
Yield — 12 mg.	
Precipitate II	**Supernatant II**
Washed with 3 volumes of 20 per cent ethanol at −5°C.	Ethanol was removed by vacuum distillation, dialyzed, and lyophilized.
Precipitate dissolved in cold water and lyophilized.	Yield — 44 mg.
Yield — 46 mg.	

The ethanol fractionation procedures employed were similar to those described by Seibert (1) for fractionating unheated tuberculin. An outline of one of the procedures which was employed to fractionate a borate buffer extract of tubercle bacilli is given in table 1.

RESULTS

Typical electrophoretic patterns of the material extracted by ether-phosphate buffer and by borate buffer may be seen in figures 1 and 2. They are similar except that they differ in the relative amounts of each component.

The average yields and analyses of material extracted by the two methods are given in table 2. As may be seen in the table, borate buffer extracted less nucleic acid and less carbohydrate from comparable cell preparations than the ether-

phosphate buffer. The activities of the material extracted by the two methods were nearly alike but the yields were significantly different. Borate buffer extracted much more material from the fresh moist cells than did ether-phosphate buffer.

FIG. 1. An electrophoretic pattern of the material extracted by ether-phosphate buffer. The pattern was obtained after 40 minutes at 5.3 volts per cm. in phosphate buffer, 0.1 ionic strength at pH 7.7. The mobility, times 10^5 cm.^{2}/volt/sec., is indicated above the component.

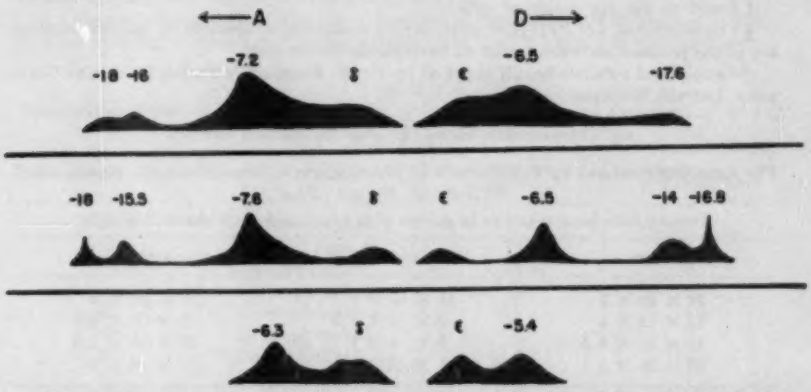

FIG. 2. (Upper) An electrophoretic pattern of material extracted from tubercle bacilli by borate buffer. The pattern was obtained after 71 minutes at 4.4 volts per cm. in phosphate buffer, 0.1 ionic strength at pH 7.7.

FIG. 3. (Center) An electrophoretic pattern of the material adsorbed by calcium phosphate from a borate buffer extract. The pattern was obtained after 75 minutes at 4.7 volts per cm. in phosphate buffer, 0.1 ionic strength at pH 7.7.

FIG. 4. (Lower) An electrophoretic pattern of the supernatant solution after removal of the calcium phosphate adsorbed material. The pattern was obtained after 67 minutes at 4.68 volts per cm. in phosphate buffer, 0.1 ionic strength at pH 7.7. The mobility, times 10^5 cm.^{2}/volt/sec., is indicated above the component.

The effect of temperature on the extraction with ether-phosphate buffer is shown in table 3. If the temperature was maintained rigorously below 0.5°C. during the entire ether treatment, the activity of the extract was appreciably higher than it was if the temperature was allowed to rise to 6°C.

In table 4, it is shown that extracts of younger cultures were more active than extracts of older cultures.

The antigenicity of 5 crude extracts are compared in table 5. The response

TABLE 2

*Analyses of Material Extracted from Tubercle Bacilli**

SOLVENT EMPLOYED	CONDITION OF CELLS	PROTEIN†	DESOXYRIBONUCLEIC ACID	CARBOHYDRATE	YIELD‡	ACTIVITY§
		per cent	*per cent*	*per cent*	*per cent*	
Ether-phosphate buffer	dried‖	30	16.3	53	2.5	7
Borate buffer only	dried‖	53	7.1	35	1.8	5
Ether-phosphate buffer	fresh	21	28	40	1.6	4
Borate buffer only	fresh	47	14.5	30	3.9	6.5

* Data based on the averages from analyses of 3 or more extractions.

† Based on the non-desoxyribonucleic acid nitrogen content of the extract. It was assumed that the desoxyribonucleic acid contained 16.7 per cent nitrogen and the protein 16.3 per cent.

‡ Based on the dry weight of cells.

§ The number of γ of PPD-S required to give a reaction comparable to that obtained by 5 γ of the preparation when tested on tuberculous guinea pigs.

‖ Lyophilized tubercle bacilli supplied by Dr. F. Stimpert of Parke, Davis and Company, Detroit, Michigan.

TABLE 3

The Reactions Produced by Two Extracts of Mycobacterium Tuberculosis var. Hominis and PPD-S in Guinea Pigs

Twenty-four-hour reaction in guinea pigs sensitized with heat-killed cells*

10γ PPD-S	10 γ EXTRACT NO. 31†	10 γ EXTRACT NO. 32‡
18 × 20 × 3	15 × 15 × 3	16 × 10 × 3
12 × 12 × 2	5 × 5 × 1.5	6 × 7 × 1.5
11 × 11 × 1.5	5 × 8 × 1	10 × 10 × 1.5
20 × 20 × 3	8 × 10 × 2	17 × 12 × 2

Twenty-four-hour reaction in tuberculous guinea pigs

5 γ PPD-S	5 γ EXTRACT NO. 31	5 γ EXTRACT NO. 32
20 × 21 × 2	10 × 10 × 1.5	13 × 14 × 2
20 × 21 × 2	13 × 14 × 2	20 × 21 × 3

* Each line indicates a different guinea pig. The two axes of the reaction were measured in mm. and the thickness recorded on a scale of 1 to 4.

† During the preparation of this extract the temperature rose to 6°C. in the presence of ether.

‡ The temperature of this preparation was not allowed to rise above 0.5°C. while ether was present in the solution.

after 8 or 11 injections of 50 γ each was not great but there were some differences between certain preparations tested. The ether-phosphate extract (No. 32) which was carefully maintained below 0.5°C. was more antigenic than the comparable extract (No. 31) which was allowed to rise to 6° C. Most, if not all, of the crude extracts were more antigenic than PPD-S.

Electrophoretic patterns of the two fractions obtained from a borate buffer extract of tubercle bacilli by calcium phosphate adsorption are given in figures 3 and 4. The calcium phosphate adsorbed all of the nucleic acid but it also removed some of the protein constituents from the supernatant solution. The mobility of the various components remained nearly constant.

TABLE 4

The Effect of the Age of the Culture on the Tuberculin Activity of the Material Extracted by Borate Buffer

Twenty-four-hour reactions in tuberculous guinea pigs*

5 γ PPD-S	3 γ EXTRACT NO. 34†	3 γ EXTRACT NO. 35‡
18 × 20 × 2	7 × 8 × 1.5	15 × 17 × 1.5
17 × 18 × 3	16 × 18 × 3	24 × 24 × 3

* Each line indicates a different guinea pig. The two axes of the reactions were measured in mm. and the thickness recorded on a scale of 1 to 4.

† A borate buffer extract of a 49-day-old culture of the TB I strain of tubercle bacilli.

‡ A borate buffer extract of a 24-day-old culture of the TB I strain of tubercle bacilli.

TABLE 5

Comparative Arthus Reactions Produced by Repeated Intracutaneous Injections of 50 γ of Various Extracts of Tubercle Bacilli into Guinea Pigs

PREPARATION NUMBER	TYPE OF EXTRACT	TOTAL NUMBER OF INJECTIONS	MAXIMUM REACTION TO LAST INJECTION*
31†	Ether-phosphate	11	1
32‡	Ether-phosphate	11	3
PPD-S	Culture filtrate	11	0
31	Ether-phosphate	8	0
32	Ether-phosphate	8	3
33	Ether-phosphate	8	2
34§	Borate buffer	8	1
35‖	Borate buffer	8	2

* Graded on a scale of 0 (no reaction) to 3 (approximately 5 mm. erythema). Usually, the reaction reached its maximum between four and twenty hours after the injection. The guinea pigs were allowed to rest two weeks before the last injection.

† The temperature rose to 6°C. during the extraction.

‡ A preparation comparable to extract No. 31 except that the temperature was maintained below 0.5°C.

§ An extract of a 49-day-old culture.

‖ An extract of a 24-day-old culture.

The activities and chemical analyses of the preparations obtained by the ethanol fractionation scheme outlined in table 1 are presented in table 6. The fractionation was not perfect, but an increase in activity was obtained in the fraction precipitated by 15 per cent ethanol at pH 5.3. Although the first precipitate contained almost as much protein as the second, the activity of the first precipitate was significantly less.

TABLE 6

*Biological Activity and Chemical Analyses of Various Fractions of Material Extracted from Tubercle Bacilli by Borate Buffer**

PREPARATION	AMOUNT INJECTED	TWENTY-FOUR-HOUR REACTION†	PROTEIN‡	CARBOHYDRATE
	γ	mm.	*per cent*	*per cent*
Extract No. 35§	5	16 × 18 × 2.5	73	36
Precipitate I (pH 5.3)	3	13 × 13 × 1.8	83	12
Precipitate II (15% ethanol at pH 5.3)	3	18 × 19 × 2.6	84	10
Supernatant from 15% ethanol at pH 5.3	3	10 × 11 × 1.4	39	53
PPD-S	2	10 × 12 × 1.8		
PPD-S	10	16 × 17 × 2.5		

* The fractionation is outlined in table 1.

† The average reaction in guinea pigs sensitized with heat-killed tubercle bacilli, produced by the intracutaneous injection of 0.1 ml. containing the indicated amount of extract.

‡ Calculated by assuming that the protein contained 16.3 per cent nitrogen.

§ A borate buffer extract after the nucleic acid was removed by calcium phosphate adsorption.

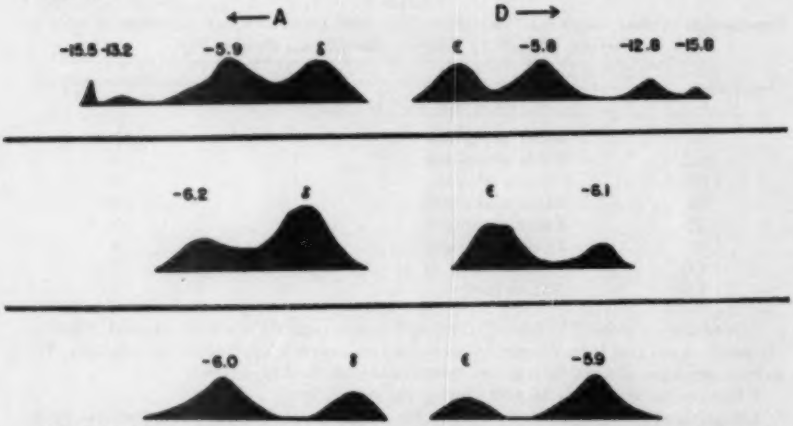

FIG. 5. (Upper) An electrophoretic pattern of a borate buffer extract of tubercle bacilli. The pattern was obtained after 45 minutes at 6.5 volts per cm. in veronal buffer, 0.1 ionic strength at pH 8.6.

FIG. 6. (Center) An electrophoretic pattern of a borate buffer extract of tubercle bacilli after removal of the nucleic acid by calcium phosphate. The pattern was obtained after 55 minutes at 6.8 volts per cm. in veronal buffer, 0.1 ionic strength at pH 8.6.

FIG. 7 (Lower) An electrophoretic pattern of the material precipitated by 15 per cent ethanol at pH 5.3 from a borate buffer extract previously freed of nucleic acid. The pattern was obtained after 105 minutes at 4.5 volts per cm. in veronal buffer, 0.1 ionic strength at pH 8.6. The mobility, times 10^5 cm.^{2}/volt/sec., is indicated above the component.

Figure 5 is an electrophoretic pattern of a crude borate buffer extract, and figure 6 is the electrophoretic pattern after the extract was treated with calcium phosphate to remove the nucleic acid prior to ethanol fractionation. Figure 7 is

the electrophoretic pattern of the final, highly active preparation (precipitate II in table 1) obtained by precipitation with 15 per cent ethanol at pH 5.3. Figure 7 shows that, although the peak produced by the protein component is

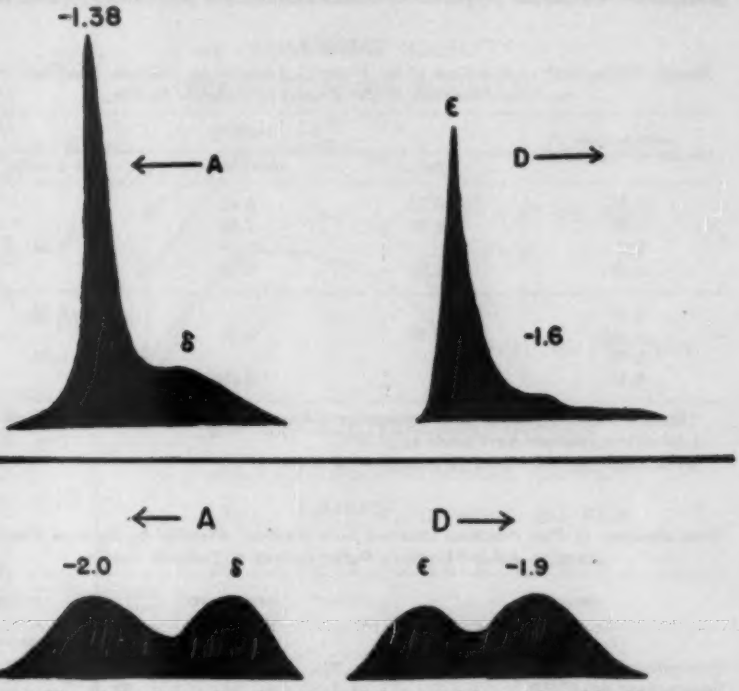

FIG. 8 (Upper) An electrophoretic pattern of the material precipitated by 20 per cent ethanol at pH 7.3 from an ether-phosphate buffer extract previously freed of nucleic acid. The pattern was obtained after 110 minutes at 5.25 volts per cm. in veronal buffer, 0.1 ionic strength at pH 8.6. The mobility, times 10^5 cm.^{2}/volt/sec., is indicated above the component.

FIG. 9. (Lower) An electrophoretic pattern of the material not precipitated by 40 per cent ethanol at pH 6.0 from an ether-phosphate buffer extract of tubercle bacilli. The pattern was obtained after 120 minutes at 6.5 volts per cm. in veronal buffer, 0.1 ionic strength at pH 8.6. The mobility, times 10^5 cm.^{2}/volt/sec., is indicated above the component.

larger, the protein is no more homogeneous than the protein component in the crude extract (figure 5).

The evidence that the component with a mobility of approximately -6×10^{-5} cm.^{2}/volt/sec. is protein is based on the correlation of the chemical and electrophoretic analyses of certain preparations. The material precipitated by 15 per cent ethanol was calculated to be 84 per cent protein (table 6), and the electro-

phoretic analyses (figure 7) indicated only one component which had a mobility of approximately -6×10^{-5} cm.^{2}/volt/sec.

The components with the low electrophoretic mobilities probably were polysaccharide because the preparations used to obtain the patterns in figures 8 and

TABLE 7

Specific Viscosities of Solutions of the Material Adsorbed by Calcium Phosphate from an Ether-Phosphate Buffer Extract of Tubercle Bacilli*

CONCENTRATION (MG. PER ML. OF SOLUTION)	SOLVENT†		
	H_2O	Borate buffer‡	0.85% NaCl
2.75	5.75	5.62	
1.37	1.88	1.60	
1.2			1.26
0.69	0.72	0.63	
0.60			0.58
0.345	0.20	0.24	
0.30			0.31
0.17		0.093	

* Specific viscosity $= \eta/\eta_0 - 1$. η = viscosity of the solution, η_0 = viscosity of the solvent.

† All determinations were made at 23.70°C.

‡ The buffer contained 0.1 M borate at pH 8.4.

TABLE 8

Some Analyses of Two Fractions Obtained from Material Adsorbed by Calcium Phosphate from an Ether-Phosphate Buffer Extract of Tubercle Bacilli

PREPARATION	ACTIVITY*	CARBOHYDRATE	DESOXYRIBONUCLEIC ACID	PROTEIN†
		per cent	*per cent*	*per cent*
Precipitated at pH 3.0	0.63	16	61	17
Supernatant at pH 3.0	1.2	75	12	15

* The average number of γ of PPD-S required to produce a reaction equivalent to that produced by 1 γ of the test preparation when tested in guinea pigs sensitized with heat-killed tubercle bacilli.

† Based on the total nitrogen after deducting the nucleic acid nitrogen. It was assumed that the desoxyribonucleic acid contained 16.7 per cent nitrogen and the protein 16.3 per cent.

9 contained approximately 98 and 86 per cent polysaccharide, respectively. The first preparation, which produced a slightly opalescent and bluish solution and was precipitated by 20 per cent ethanol at pH 7.3, corresponds to Seibert's polysaccharide II (1). The other polysaccharide probably is equivalent to Seibert's polysaccharide I because it is water clear and soluble in 40 per cent ethanol. Both of the polysaccharides appear to have mobilities between -1 and -2×10^{-5} cm.^{2}/volt/sec., which is in agreement with the mobilities reported by Seibert for her polysaccharides I and II.

Solutions of the material adsorbed by calcium phosphate from crude extracts of tubercle bacilli were extremely viscous (table 7). The high specific viscosity of the preparations which have been examined was not dependent on the solvent since approximately the same viscosity was observed in 0.85 per cent sodium

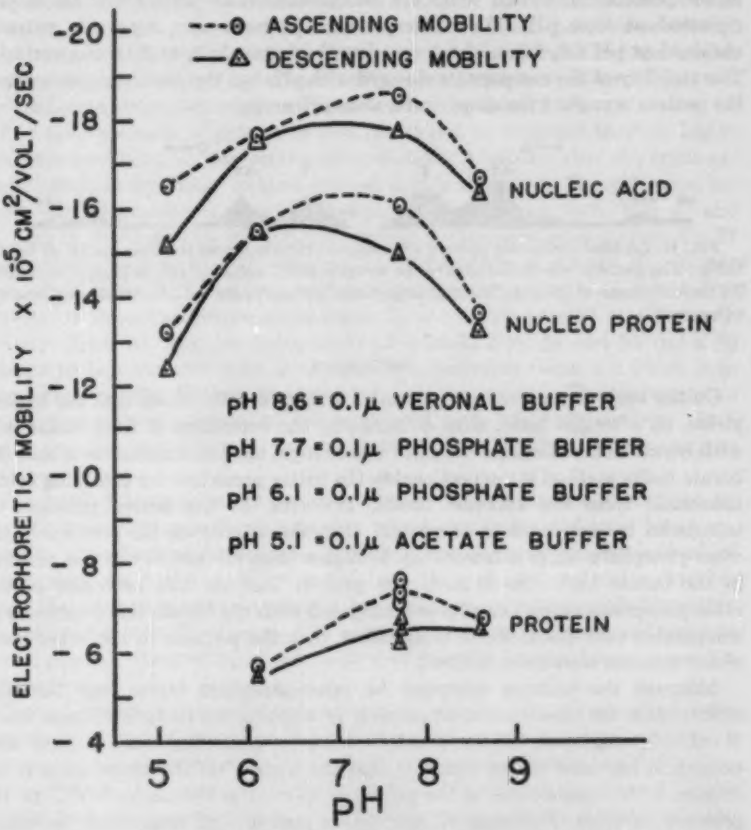

FIG. 10. Electrophoretic mobility curves of the major components of extracts of tubercle bacilli.

chloride, in 0.1 M borate buffer at pH 8.4, and in distilled water. The nucleic acid, therefore, must have been highly polymerized.

The analyses of fractions obtained by acid precipitation of a crude nucleic acid preparation are given in table 8. Most of the nucleic acid was precipitated at pH 3.0 but a large amount of protein and polysaccharide also was precipitated. No precipitate was formed until the pH reached 3.5; and, also, when the

pH was lowered to 2.8, some of the precipitate was redissolved. Therefore, pH 3.0 probably was the optimum pH to obtain the maximum precipitate.

The electrophoretic mobilities of three major components extracted from tubercle bacilli by borate buffer are plotted against pH in figure 10. The mobility of the protein component could not be determined at pH 5.1 because it precipitated at that pH. The electrophoretic patterns of comparable extracts obtained at pH 8.6, 7.7, and 6.1 are given in figures 5, 2, and 11, respectively. The mobility of the components changed with pH, but the general appearance of the pattern remained the same over a wide pH range.

FIG. 11. An electrophoretic pattern of material extracted from tubercle bacilli by borate buffer. The pattern was obtained after 60 minutes at 4.7 volts per cm. in phosphate buffer, 0.1 ionic strength at pH 6.1. The mobility, times 10^5 cm.^{2}/volt/sec., is indicated above the component.

DISCUSSION

On the basis of the data given in table 2 there is little doubt that the highest yields, on a weight basis, were obtained by the extraction of fresh moist cells with borate buffer. Because the yield was so high, the first impression is that the borate buffer method is unquestionably the better procedure for obtaining active tuberculin from the tubercle bacilli. However, as the active principle of tuberculin is considered to be protein (12), the activity of the proteins in the ether-phosphate buffer extracts may be higher than the activity of the proteins in the borate buffer extracts. If the protein analyses and activities of the ether-phosphate buffer extracts are compared with the borate buffer extracts of comparable cells (table 2), it is apparent that the proteins in the ether-phosphate extracts were more active.

Although the proteins extracted by ether-phosphate buffer may be more active, there are other factors to consider in selecting the buffer for future work. If ether is employed, the temperature must be controlled with extreme care because it has been shown (table 3) that the activity of the preparation is decreased if the temperature of the extract is allowed to rise above 0.5°C. in the presence of ether. Furthermore, the higher nucleic acid content of the ether-phosphate buffer extract imposes additional problems in the fractionation of the extracts.

It was not expected that the nucleic acid content of the borate buffer extract would be lower than that of the ether-phosphate buffer extract because Chargaff and Saidel (4) had used a borate buffer to extract nucleoproteins from tubercle bacilli. A possible explanation is that the avian tubercle bacilli, with which Chargaff worked, are different from the mammalian type of tubercle bacilli with respect to the nature of the nucleic acid complexes. Chargaff had not applied an ether-phosphate buffer to the extraction of avian tubercle bacilli, however, and

it is possible that the borate buffer is not the best solvent for extracting nucleoproteins.

Since the two methods of extracting tubercle bacilli appeared to yield extracts of such different chemical composition, it was surprising that the typical electrophoretic patterns of two extracts (figures 1 and 2) should be so similar. Apparently, the same major components were extracted, and the extracts differed only in the relative concentrations of the various constituents. Perhaps further study and fractionation of the extracts will reveal the presence of several proteins with different properties, such as those identified by Seibert (1).

The mild methods of extracting proteins might be expected to yield highly antigenic materials, but the results given in table 5 indicate that the crude extracts obtained by either method are not highly antigenic. The difference between the antigenicity of the two ether-phosphate buffer extracts, Nos. 31 and 32, may be attributed to the fact that during the preparation of extract No. 31 the temperature rose to 6°C., whereas the temperature of extract No. 32 was maintained below 0.5°C. as long as the extract was in contact with ether. The activity of these two preparations (table 3) is roughly parallel to their antigenicity (table 5). Also the antigenicity of extracts Nos. 34 and 35 (table 5) appears to be correlated with their tuberculin activities (table 4), which is in agreement with the findings of McCarter and Watson (13). They have shown that antigenicity of tuberculins was correlated with tuberculin activity. Consequently, any very active fraction extracted from tubercle bacilli also may be more antigenic.

The small differences in antigenicity between the crude extracts obtained by the two extraction procedures probably is not significant and may be an expression of individual variations. If rabbits had been employed instead of guinea pigs, the Arthus reaction would have been greater and differences between the various preparations might have been more pronounced. Nevertheless, McCarter and Bevilacqua (14) were able to obtain sizable reactions in guinea pigs by repeated injections of 50 γ of proteins obtained from unheated culture filtrates.

Three different protein components can be identified in the electrophoretic patterns of unheated tuberculin (1), but only one protein component has been observed in the electrophoretic patterns of extracts of tubercle bacilli after removal of the nucleic acid. The fact that Seibert (1) isolated the three different proteins is ample proof that they are distinct entities and not anomalies in the electrophoretic patterns. It should be pointed out, however, that the protein in the extracts of tubercle bacilli is not electrophoretically homogeneous because a large amount of reversible spreading of that component occurred during electrophoresis.

Often it is possible, by changing the pH of the buffer, to separate an apparently homogeneous preparation into two or more components. It is evident from figures 2, 5, and 11 that the component with a mobility of -5.8×10^{-5} cm.^{2}/volt/sec. at pH 8.6 is not resolved readily into more than one component. Furthermore, in none of the electrophoretic patterns of the various fractions has there been any evidence that there is more than one protein.

The nature of the component in the whole extracts, which has been observed to migrate immediately after the nucleic acid, has been discussed previously (3) and it has been identified tentatively as a nucleoprotein. Some electrophoretic patterns of material adsorbed by calcium phosphate show the nucleoprotein component (figure 3), but patterns of other preparations treated with calcium phosphate do not show the component in either the supernatant or precipitated portion of the crude extract (3). The fate of the nucleoprotein remains to be determined. It is not certain whether the protein, originally combined with the nucleic acid in the crude extracts, is different from the major protein component.

It is difficult, without further study, to explain adequately the discrepancy between the number of proteins in the culture filtrates and in the cell extracts. One explanation is that the three proteins in the unheated culture filtrates are produced by autolytic processes on the single protein in the cells, but it is as likely that the extraction of the tubercle bacilli with a buffer is very specific. The agents used to extract the cells were relatively mild, and it may be that enzymatic action is required to separate the other proteins from the cell debris.

There is evidence that there was more than one protein present in the crude extracts although the electrophoretic analyses indicated that only a single protein was extracted from the tubercle bacilli. If there were only one protein, the tuberculin activity of the different fractions of the extracts would be correlated with the protein content because it has been established that the active principle in tuberculin (12) is protein. In table 6 it is shown that the protein content of precipitates I and II was nearly identical, but the activity of precipitate I was much less than that of precipitate II; therefore, there must be at least two proteins with different tuberculin activities. Seibert (1) designated the protein which she precipitated from culture filtrates at pH 3.8 to 4.7 as protein C. The other two proteins, which she designated as A and B, were precipitated with ethanol at pH 4.6, and she reported that the tuberculin activity of her protein C was considerably less than that of the other proteins. Precipitate I (table 6) resembles Seibert's protein C in that it was precipitated without ethanol at pH 5.3 and it has a lower activity than precipitate II.

The major problem in the fractionation of extracts of tubercle bacilli, which is not encountered in the fractionation of culture filtrates, is the removal of the extremely viscous nucleic acid. Heckly and Watson (3) showed that it is possible to remove all of the nucleic acid, or to remove only nucleic acid from the crude extracts by adsorption with calcium phosphate. Although adsorption with calcium phosphate is a convenient method, it is not entirely satisfactory because it is rather difficult to remove all of the nucleic acid without also removing some protein from the crude extract. Figures 3 and 4 are electrophoretic patterns of the preparations obtained by calcium phosphate adsorption under such conditions that all of the nucleic acid was removed. It is clear that an appreciable amount of protein also was adsorbed by the calcium phosphate. As the pH was lowered, there was probably a greater interaction between the nucleic acid and the protein but, also, it is possible that it was the calcium phosphate which adsorbed the protein in more acid solutions.

By adjusting the pH of a solution of the material adsorbed by calcium phosphate to pH 3.0, most of the nucleic acid was precipitated but, as is shown in table 8, a large amount of protein also was precipitated. It is of interest that the activities of the two preparations obtained by acid fractionation of the nucleic acid rich preparation should differ by so much and yet contain nearly the same amount of protein. This evidence can be considered to be further proof that there is more than one protein in the extracts obtained from tubercle bacilli. Again the protein with the least activity was precipitated from the acid solution, indicating that it may be similar to Seibert's C protein.

Only ethanol fractionation has been employed to separate the various constituents of extracts of tubercle bacilli after the preliminary adsorption with calcium phosphate because the use of ethanol has been so successful in the fractionation of serum proteins. The ethanol fractionation procedure outlined by Seibert (1) was not particularly well suited to the fractionation of the extracts, but her procedures were used as a guide for the preliminary experiments. It was found that the active protein could be precipitated from a 15 per cent ethanol solution without lowering the pH below 5.3. Furthermore, it was not necessary to first remove the polysaccharide which she designated polysaccharide II. It was shown that this polysaccharide could be removed from the cell extracts, however, by precipitation with 30 per cent ethanol, as described by Seibert. It is possible that better conditions can be found which will effectively separate the active protein from the other constituents of extracts of tubercle bacilli because there are so many independent variables which may be controlled when ethanol is employed for fractionation.

In addition to the main objective of obtaining a more specific agent for tuberculin testing, the present studies may also provide means of obtaining better antigen preparations for use in serologic tests, such as those now being employed for the diagnosis of tuberculosis (15, 16, 17). The use of crude Old Tuberculin may account for some discrepancies in the results of the hemagglutination test being performed in the various laboratories.

In a recent paper, Middlebrook (18) has described a hemolytic modification of his original hemagglutination test. Although both the original hemagglutination test and the newer hemolytic modification appear to be specific, they do not measure the same antibodies. Perhaps purified antigens, extracted from the cells by mild procedures, may be used to determine how the two tests are related.

SUMMARY

It was found that, when mechanically ruptured tubercle bacilli were extracted with a 0.1 M borate buffer at pH 8.4, more material was rendered water soluble than when the cell debris was extracted with a phosphate buffer containing ether. Both procedures yielded extracts containing essentially the same substances but in different proportions. Borate buffer extracted less carbohydrate and nucleic acid but more protein than did the ether-phosphate buffer. The average tuberculin activity of the crude extracts was comparable to that of purified protein derivative (PPD).

There was evidence that the average potency of the proteins in the ether-phosphate buffer extracts was greater than that of the proteins in the borate buffer extracts. However, the temperature had to be maintained below 0.5°C. during the extraction to prevent denaturation of the proteins by ether.

The electrophoretic mobilities of the various components in borate buffer extracts were nearly identical with the mobilities of corresponding components in either-phosphate buffer extracts. Also the general appearance of the electrophoretic patterns of a crude borate buffer extract of tubercle bacilli was the same at pH 8.6, 7.7, and 6.1.

It was found that the crude extract of a young culture had more tuberculin activity than a comparable extract of an older culture of tubercle bacilli.

Problems involved in the removal of nucleic acid from extracts of tubercle bacilli have been considered. Extracts have been obtained free of nucleic acid by adsorption with calcium phosphate, but some protein also was removed from the extracts by the procedure. The nucleic acid obtained from the crude extracts by calcium phosphate adsorption is highly polymerized.

Fractionation procedures employing ethanol were applied to the extracts after the nucleic acid was removed by calcium phosphate adsorption. One fraction, precipitated by 15 per cent ethanol at pH 5.3, contained 84 per cent protein and was approximately three times as active as PPD-S. Another protein fraction was obtained which had properties similar to protein C, isolated by Seibert from unheated culture filtrates. Two polysaccharides which corresponded to the polysaccharides designated I and II by Seibert also were obtained in a relatively pure form by ethanol fractionation.

Only a single protein component was evident by electrophoresis, but the correlation of activity and protein content of various fractions indicated the presence of two or more proteins in the extracts of tubercule bacilli. These proteins differed primarily in their tuberculin activity.

SUMARIO

Extracción y Fraccionación de los Componentes Hidrosolubles de los Bacilos Tuberculosos

Al extraer con un amortiguador de borato de 0.1 M a un pH de 8.4 bacilos tuberculosos fragmentados mecánicamente, más material se volvió hidrosoluble que cuando los desechos celulares eran extraídos con un amortiguador de fosfato que contenía éter. Ambos procedimientos rindieron extractos que contenían esencialmente las mismas substancias, pero en distintas proporciones. El amortiguador de borato extrajo menos hidratos de carbono y ácido nucleico pero más proteína que el de fosfato-éter. La actividad tuberculínica media de los extractos crudos era comparable a la del derivado proteínico purificado (PPD).

Había pruebas de que la potencia media de las proteínas en los extractos obtenidos con el amortiguador de fosfato-éter era mayor que la de las contenidas en los extractos obtenidos con el amortiguador de borato. Sin embargo, había que mantener la temperatura por debajo de 0.5°C. durante la extracción a fin de impedir la desnaturalización de las proteínas por el éter.

Las movilidades electroforéticas de los varios componentes de los extractos boratados fueron casi idénticas a las de los correspondientes componentes en los extractos amortiguados con fosfato-éter. Además, no varió el aspecto general de los patrones electroforéticos de un extracto crudo de bacilos tuberculosos amortiguado con borato ya fuere el pH de 8.6, 7.7, ó 6.1.

Observóse que el extracto crudo de un cultivo joven mostraba más actividad tuberculínica que un extracto comparable de un cultivo tuberculoso más viejo.

Los problemas comprendidos en la separación de ácido nucleico de los extractos de bacilos tuberculosos recibieron atención. Obtuviéronse extractos exentos de ácido nucleico mediante la adsorción con fosfato de calcio, pero también se eliminó así alguna proteína de los extractos. El ácido nucleico obtenido de los extractos crudos por adsorción con fosfato de calcio está altamente polimerizado.

Después de separar el ácido nucleico por adsorción con fosfato de calcio, se aplicaron a los extractos procedimientos de fraccionación que utilizaban etanol. Una fracción, precipitada por 15 por ciento de etanol a un pH de 5.3, contenía 84 por ciento de proteína y mostró una actividad aproximadamente triple que la del PPD-S. Obtúvose otra fracción proteínica dotada de propiedades semejantes a las de la proteína C, aislada por Seibert de filtrados de cultivos no calentados. Los polisacáridos correspondientes a los denominados I y II por Seibert fueron también obtenidos en forma relativamente pura por la fraccionación con etanol.

La electroforesis no puso de manifiesto más que una sola proteína, pero la correlación de la actividad y del contenido proteínico de varias fracciones indicó la presencia de dos o más proteínas en los extractos de bacilos tuberculosos. Esas proteínas variaban primordialmente en su actividad tuberculínica.

RESUME

Extraction et fractionnement d'élements, solubles dans l'eau, composant le bacille de Koch

Il a été observé que, lorsque le bacille tuberculeux était rompu mécaniquement, l'extraction dans une solution-tampon boratée à 0,1 M de pH 8,4, permettait d'obtenir plus de matière que lorsque les eléments cellulaires étaient extraits en utilisant une solution-tampon phosphatique contenant de l'éther. Ces deux procédés ont donné des extraits contenant essentiellement les mêmes substances, mais en proportions différentes. L'extraction par la solution-tampon boratée donnait moins d'hydrate de carbone et d'acide nucléique, mais plus de protéine que la solution-tampon phosphate-éther. L'activité tuberculinique moyenne de l'extrait brut était comparable à celle du dérivé protéique purifié (PPD).

Il a été constaté que l'activité moyenne des protéines des extraits de la solution-tampon phosphate-éther était plus élevée que celle des protéines des extraits de la solution-tampon boratée. Pour prévenir la dénaturation des protéines par l'éther pendant l'extraction, la température doit être maintenue au-dessus de 0,5°C.

La mobilité électrophorétique des divers constituants des extraits de la solution-tampon boratée était sensiblement la même que celle des composants des

extraits de la solution-tampon phosphate-éther. L'aspect général du type électrophorétique d'un extrait brut de bacilles tuberculeux de la solution-tampon boratée était le même aux pH 8,6, 7,7, et 6,1.

Il a été observé que l'activité tuberculinique de l'extrait brut d'une culture jeune était plus grande que celle d'un extrait comparable d'une culture plus vieille.

Les problèmes que soulève l'extraction de l'acide nucléique, à partir d'extraits de bacilles tuberculeux, ont été considérés. Des extraits débarrassés de l'acide nucléique ont été obtenus par adsorption dans le phosphate de calcium, mais ce procédé entraîne une perte de protéine. L'acide nucléique obtenu à partir d'extraits bruts, par adsorption dans le phosphate de calcium, est largement polymérisé.

Le procédé de fractionnement à l'aide de l'éthanol a été appliqué aux extraits, après que l'acide nucléique ait été enlevé par adsorption dans le phosphate de calcium. Une fraction précipitée par l'éthanol à 15%, au pH 5,3, contenait 84% de protéine et son activité était trois fois plus grande que le PPD-S. Une autre fraction protéique obtenue possédait des propriétés analogues à celles de la protéine C, isolée par Seibert à partir de filtrats de culture non chauffés. Deux polysaccharides, correspondant aux polysaccharides I et II de Seibert, ont également été obtenus sous une forme relativement pure par fractionnement à l'éthanol.

Un seul composant protéique a été décelé par l'électrophorèse, mais la corrélation entre l'activité et la valeur protéique des diverses fractions indiquait la présence de deux ou plusieurs autres protéines dans les extraits de bacilles tuberculeux. Les protéines différaient surtout dans leur activité tuberculinique.

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PULMONARY TUBERCULOSIS, BRONCHIECTASIS, AND CALCIFICATION AS RELATED TO BRONCHOGENIC CARCINOMA[1]

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INTRODUCTION

For many years the relationship between tuberculosis and primary carcinoma of the lung has intrigued pathologists and clinicians alike. Nearly 100 years ago the published work of Von Rokitansky (1) gave rise to the theory that tuberculosis and cancer are antagonistic. Actually, Von Rokitansky was concerned chiefly with the varied organ incidence of the two conditions. He pointed out the relatively large number of cases of pulmonary tuberculosis and the rarity of carcinoma of the lung; the fact that, in the ovary, cancer is common and tuberculosis rare; and that tuberculosis of the epididymis is common, while in the testicle carcinoma is much the commoner lesion. He states further (1) that "Whenever their general correlation (of observations concerning tuberculosis and carcinoma) is susceptible of proof, cancer has seemed to succeed to tuberculosis, tubercle rarely to become developed after the extinction of cancer and its crasis."

The question of a possible relationship between tuberculosis and carcinoma was given renewed interest by Pearl's study (2) of 6,670 post-mortem records accumulated at the Johns Hopkins Hopsital. Included in the study were the records of 886 persons with active, florid tuberculosis. These were compared with the records of 886 deceased persons of the same age, sex, and racial distribution but with no recorded lesions of tuberculosis at autopsy. In the first group, only 11 cases (1.2 per cent) of malignant tumors were found while in the second group there were 82 cases (9.3 per cent) of malignant tumors. Pearl concluded that "... there is a definite and marked incompatibility or antagonism between the two diseases, carcinoma and tuberculosis, of such sort that both occur together at the same time in florid activity in the same individual only with great rarity."

Carlson and Bell (3), after an analysis of 11,195 post-mortem protocols, including 1,184 cases of cancer, agreed that active tuberculosis is much less frequent in cancerous than in noncancerous subjects, but they found no statistical evidence to support the concept of an actual antagonism between cancer and tuberculosis, a view subscribed to by Cooper (4).

The studies mentioned above included malignant tumors of all the viscera. With regard to the lung alone, the past few years have brought an increasing number of reports concerning the simultaneous occurrence of tuberculosis and bronchogenic carcinoma. Among them are those of Fried (5), Gerstl, Warring, and Howlett (6), Drymalski and Sweany (7), Shefts and Hentel (8), Phillips and Morrison (9), and Moyer and Ackerman (10). This increase in the number of articles on associated active pulmonary tuberculosis and carcinoma is probably

[1] From the William H. Maybury Sanatorium (Detroit Municipal Tuberculosis Sanatorium), Northville, Michigan.

due to the increased number of such cases screened out of the general population by the large number of roentgenographic surveys currently in progress in the United States.

From the long-range point of view it would seem that, as was stated by Pearl, one may expect to find tuberculosis and carcinoma "together at the same time in florid activity in the same individual" only rarely.

Weller (11), after a careful study of primary carcinoma of the lung, accepted the conclusion of Kikuth (12) that: "... tuberculosis plays a small role, if any, in determining a malignant pulmonary condition, occupying in this respect exactly the same position as a considerable number of other chronic inflammatory diseases." Graham (13), likewise, stated that: "In our own experience this association (of pulmonary cancer and tuberculosis of the lung) has been rare in a fairly large number of cases of primary carcinoma of the lung."

While the incidence of bronchogenic carcinoma in individuals with active pulmonary tuberculosis may be the same as, or less than, that in nontuberculous individuals, the same does not necessarily hold with regard to healed tuberculosis. Jackson (14) has observed bronchogenic carcinoma in patients with calcification of the tracheobronchial lymph nodes. Furthermore, Steiner and his associates (15), while using a digestion technique to identify calcified foci in the human lung, studied three cases of primary carcinoma in which the right and left lungs were analyzed separately. In each of these cases the lung with tumor was found to contain focal calcium while none was recovered from the opposite lung. Three cases, obviously, are insufficient for a statistical analysis, and Steiner and his associates attempted none. Nevertheless, the finding is suggestive. Large calcified foci in the lungs or tracheobronchial lymph nodes usually may be considered as milestones marking an area of healed primary tuberculosis of former years. One must consider the possibility that the stones may also mark a pulmonic area which is particularly susceptible to the development of bronchogenic carcinoma.

Stimulated by the paper of Steiner and his associates, as well as by casual observations made in the Maybury Sanatorium laboratory during the past several years, the decision was reached to study all of the available material in order to determine in a larger series of cases whether or not pulmonary calcification and bronchogenic carcinoma are closely associated.

MATERIAL AND METHODS

Post-mortem material available for analysis consisted of the protocols and associated roentgenograms of 1,144 autopsies performed at the Wm. H. Maybury Sanatorium during the past twenty-five years. A survey of this material revealed 15 cases of primary carcinoma of the lung. In addition, 4 cases of bronchogenic carcinoma were found in which the type of tumor was diagnosed following bronchial biopsy, and 4 cases diagnosed following lobectomy or pneumonectomy. One case each of lymphoblastoma, pulmonary adenomatosis, and teratoma primary in the lung and 2 cases of pleural endothelioma have been excluded from the series.

As already mentioned, the object of the study was to determine whether or not calcified foci mark a pulmonic area particularly susceptible to the development of carcinoma. Such foci were considered to be "closely associated" with the tumor only if they were found in the same lobe or in the tracheobronchial lymph nodes draining that lobe. Furthermore, only those calcified foci of such a size and anatomic distribution as to suggest healed tuberculosis were considered. Accordingly, an average diameter of 5 mm. or more was set for the calcified foci. Foci smaller than this were not included in the final tabulation of data.

TABLE 1

The Association of Calcified Foci and Bronchogenic Carcinoma

CASE NUMBER	COLOR	SEX	AGE	LOBE	ACTIVE TUBERCULOSIS	CALCIFIED FOCUS ASSOCIATED WITH TUMOR	HISTOLOGIC DIAGNOSIS
1	W	M	59	Upper	—	+	S*
2	W	M	54	Upper	—	+	S
3	W	M	55	Upper	—	+	S
4	W	M	48	Upper	—	+	S
5	W	M	61	Upper	C†	+	S
6	W	M	81	Upper	—	+	S
7	W	M	65	Upper	—	+	S
8	W	M	45	Upper	—	+	S
9	W	M	60	Upper	—	+	S
10	W	M	61	Upper	—	—	S
11	W	M	62	Upper	C	+	S
12	W	M	55	Upper	—	+	S
13	W	M	68	Upper	—	+	U‡
14	W	M	60	Lower	C	—	U
15	W	M	57	Upper	—	—	U
16	W	M	42	Lower	—	—	U
17	W	M	54	Middle	—	—	U
18	W	M	87	Lower	+	+	U
19	W	M	60	Lower	—	+	U
20	W	M	65	Lower	+	—	U
21	W	M	64	Upper	—	—	U
22	W	M	45	Lower	—	—	A§
23	N	M	51	Lower	+	—	A

* S—Squamous Cell

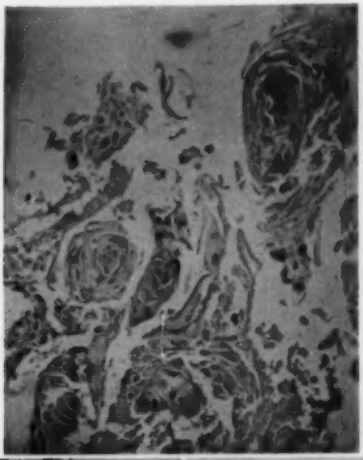
† C—Culture positive for *M. tuberculosis*

‡ U—Undifferentiated

§ A—Adenocarcinoma

Early in the course of the study it was suggested that the findings relative to healed tuberculosis might vary in the different histologic types of primary carcinoma of the lung. For this reason the histologic sections were carefully reviewed to determine in each particular case whether it should be classified as adenocarcinoma, as an undifferentiated type of lung tumor, or as squamous cell carcinoma. No case was included in the latter category unless definite epithelial pearls could be demonstrated in the sections.

The lobe primarily involved by tumor was determined from the autopsy protocol or from the description of the surgical specimen, if available; otherwise from the chest roentgenogram and the report of the bronchoscopist.



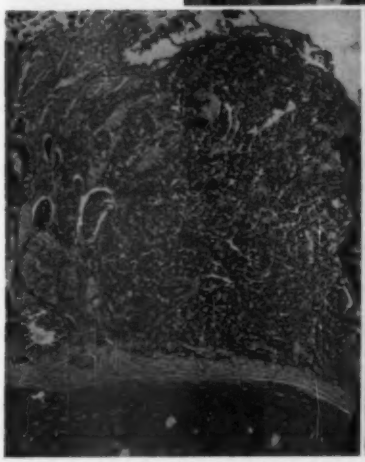


FIG. 1. Case 7. Epithelial pearls and epithelial debris lining central cavity of squamous cell carcinoma. × 220.

FIG. 2. Case 21. Undifferentiated carcinoma. The entire mucosa, from cartilage to basement membrane, is densely infiltrated with small, undifferentiated cells. × 70.

FIG. 3. Case 23. Group of cells from smear of bronchial aspiration. This case was diagnosed as adenocarcinoma following resection of the left lower lobe. × 1200.

In view of the current interest in tobacco smoke as a possible etiologic factor in the production of bronchogenic carcinoma, the clinical chart of each patient was reviewed in an attempt to determine whether or not he had been a smoker of tobacco. Information bearing on this particular point was available in only 17 of the 23 histories, in every case in the affirmative. The only case of a definite nonsmoker was the patient with pulmonary adenomatosis mentioned in the first paragraph of this section.

RESULTS

Results of the study are shown in table 1. There were 12 cases of squamous cell carcinoma identified by the finding of definite epithelial pearls in the sections (figure 1). Nine cases were classified as undifferentiated in type (figure 2). Two cases of adenocarcinoma were found (figure 3). As indicated in the table, large calcified foci were found closely associated with the tumor in 91.5 per cent (11 of 12) of the cases of squamous cell carcinoma. These calcified foci were of such a size and roentgenographic appearance (figure 4) that their age would be estimated (16) at many years, long antedating the usually accepted duration of carcinoma of the lung.

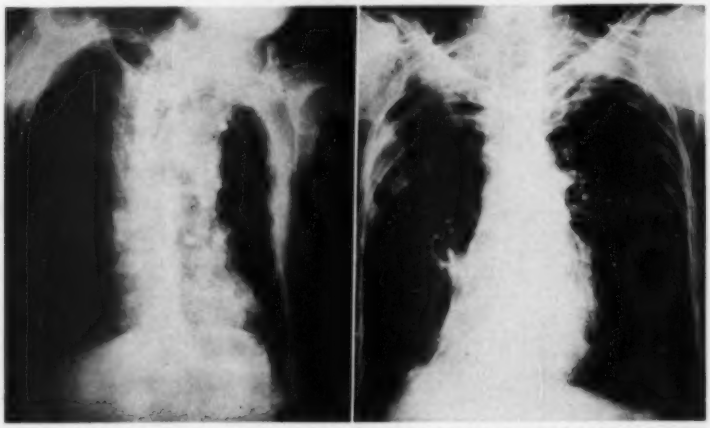


FIG. 4. (Left) Case 6. Roentgenogram showing calcified foci associated with squamous cell carcinoma of left upper lobe.

FIG. 5. (Right) Case 7. Squamous cell carcinoma in right upper lobe. Calcified tracheobronchial lymph nodes. Roentgenogram taken September 13, 1950.

One of these cases is of particular interest. The history was obtained of pulmonary hemorrhages in 1906 when the patient was twenty years of age. A roentgenogram taken in 1942 showed large calcium deposits, especially in the tracheobronchial lymph nodes. Eight years later the first roentgenographic evidence of carcinoma of the lung was obtained (figure 5). The final ante-mortem film, taken two months before death, showed a large growth (figure 6), revealed in more detail by a post-mortem film of a section of the lung (figure 7).

In only 3 of the 9 cases of undifferentiated carcinoma were large calcified foci found closely associated with the tumor. In these cases there was also extensive calcification in other portions of the lung (figures 8 and 9). In 4 of the remaining cases of undifferentiated carcinoma, large calcified foci were found in a different lobe of the lung from that occupied by the tumor. Neither of the 2 cases of adenocarcinoma showed close association of calcified foci and tumor.

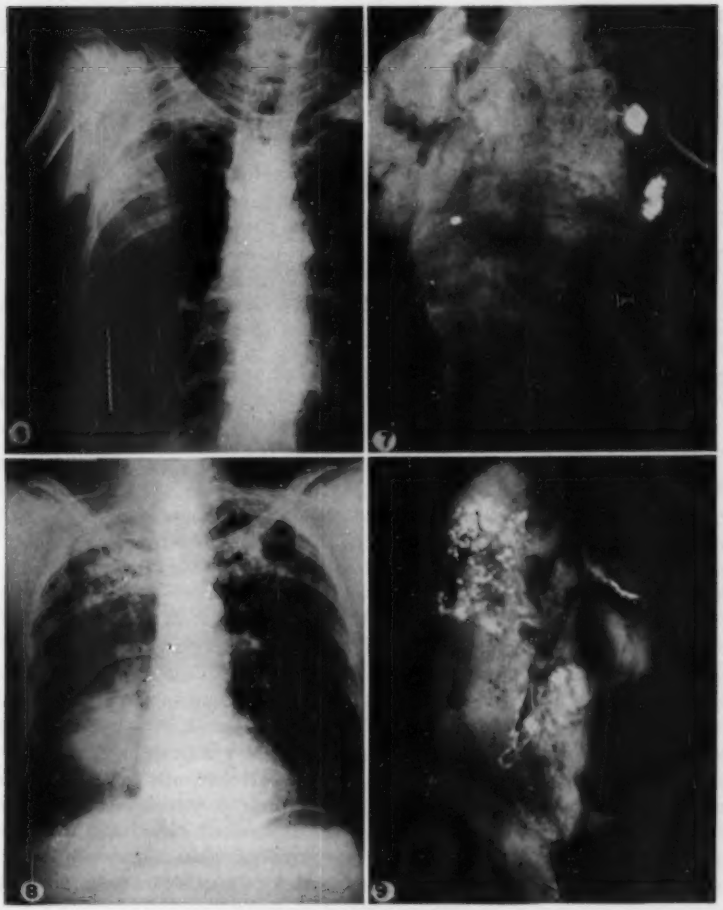


FIG. 6. Case 7. Roentgenogram taken December 5, 1950.

FIG. 7. Case 7. Post-mortem roentgenogram of a 2 cm. section through right lung. Central excavation of tumor mass is shown as well as involvement of portions of two ribs. Tracheobronchial lymph nodes contain calcium but no tumor.

FIG. 8. Case 18. Roentgenogram showing undifferentiated carcinoma of right lower lobe.

FIG. 9. Case 18. Post-mortem roentgenogram of a 2 cm. section through right lung. There is extensive calcification in the right upper lobe as well as in nodes about the right lower lobe bronchus. The tracheobronchial lymph nodes are swollen with tumor cells.

With regard to age, sex, and color it will be noted that all of the cases were in males and that 22 of the 23 cases were in white men. The youngest patient in the

series was forty-two years of age; 19 of the 23 patients were fifty years of age or older.

All of the squamous cell carcinomata were found in the upper lobe, about evenly divided between right and left lungs. The undifferentiated type was found in both upper and lower lobes, with one case primary in the middle lobe. Both cases of adenocarcinoma were found in the lower lobe.

The sputum of each patient had been investigated carefully for the presence or absence of tubercle bacilli. Evidence of active tuberculosis was found in only 2 of the 12 cases of squamous cell carcinoma and then only after the sputum had been concentrated and cultured. None of these 12 cases showed acid-fast bacilli in smears of the sputum. Three of the 9 cases of undifferentiated carcinoma, on the other hand, showed associated active tuberculosis; in the 2 instances indicated by "+", acid-fast bacilli were found in direct smears of the sputum. Numerous acid-fast bacilli were found in the sputum of one of the 2 cases of adenocarcinoma.

DISCUSSION

The data in the present series of bronchogenic tumors suggest that the squamous cell carcinomata, identified histologically by the finding of epithelial pearls, have certain associated anatomic and bacteriologic characteristics: (*1*) They are rarely associated with active tuberculosis; (*2*) they are found most commonly in the upper lobe; and (*3*) they are frequently associated with large calcified foci in the tracheobronchial lymph nodes.

The failure to find tubercle bacilli in the sputum smears of any of the patients with squamous cell carcinoma of the lung seemed surprising, particularly since obvious central excavation of the tumor was present in most cases. Moreover, most of the men had been exposed to open cases of tuberculosis during their stay in the sanatorium, if not before. As shown in figure 1, however, the central cavity of a tumor of this type is lined by the debris from squamous epithelial cells, and squamous epithelium is notoriously resistant to infection with tubercle bacilli. It will be interesting to see whether this differing incidence of active tuberculosis will be found in other series of bronchogenic carcinomata when the criterion of epithelial pearls is used for differentiating the squamous cell type.

The predilection of squamous cell carcinoma for those areas of the lung which are the seat of a *healed* tuberculous process prompts the question, "why"? One obvious possible explanation is that a common residuum of tuberculous infection is bronchiectasis (17, 18, 19) and that in an area of old bronchiectasis the normal columnar epithelium of the bronchus is frequently transformed into epithelium of the stratified squamous variety (20, 21). This type of epithelial metaplasia, the result of chronic irritation, usually precedes the development of squamous cell carcinoma in any of the viscera, whether gall bladder, genitourinary tract, or lung (22).

The much more frequent occurrence of squamous cell carcinoma in the upper lobes (5, 23) may be associated with the fact that saccular bronchiectasis is found more commonly in the upper lobes, cylindrical bronchiectasis being the

usual lesion in the lower lobes (21). Moreover, it is well known that the wall of an old tuberculous cavity may eventually become completely covered with squamous epithelium (24), and several cases have been noted of epidermoid carcinoma arising directly in the wall of such a tuberculous cavity (25, 26).

Bronchiectasis has long been recognized as a common finding in primary carcinoma of the lung but has usually been considered a *result* of the bronchial obstruction caused by the tumor. The finding of calcified lymph nodes closely associated with bronchogenic carcinoma means that in these cases it is possible, if not probable, that bronchiectasis *preceded* the development of the tumor.

Experimental work on the etiology of malignant disease has shown the importance of specific carcinogenic agents; in the lung the role played by inhaled dusts has been demonstrated in the well-known cancers of Schneeberg, Saxony, and Joachimstal, Bohemia. Of more general importance, it has been shown that the tar derived from tobacco smoke contains a benzpyrene which is definitely carcinogenic (27), and there is much statistical evidence (28, 29) that inveterate smoking may be an important factor in the causation of bronchogenic carcinoma.

Although the carcinogenic effect of tobacco smoke tar has been demonstrated, still it is difficult to conceive of sufficient tobacco tar accumulating in the normal bronchial tree to give rise to a tumor. In an area of saccular bronchiectasis, on the other hand, such an accumulation of tar might occur much more readily.

Wynder and Graham (28) state that:

> Since in a small percentage of cases cancer of the lungs occurs in nonsmokers and minimal smokers and since it obviously does not develop in every person who has been a heavy smoker for a long time, it is apparent that smoking cannot be the only etiologic factor in the induction of the disease.

The present study suggests that an associated etiologic factor of importance may be healed tuberculosis which has left behind an area of bronchiectasis in the lungs and a calcified focus in the adjacent lymph nodes. In other words, one would conclude that a person in the "cancer age" might have smoked for many years with little danger of developing pulmonary malignancy if there were no associated bronchiectasis. On the other hand, a person with bronchiectasis would run little danger of malignant change occurring if he were a nonsmoker. A combination of the two conditions, bronchiectasis and inveterate smoking, however, would compound the chances of malignancy developing in the lung.

SUMMARY

A series of 23 cases of bronchogenic carcinoma included 12 of the squamous cell type sufficiently differentiated to form epithelial pearls. All 12 of this variety were found in either the right or left upper lobe; all but one were found associated with large calcified foci in the same lobe or in the adjacent tracheobronchial lymph nodes; and all but 2 showed no associated active tuberculosis. Lung tumors of other types showed quite different anatomic findings.

An explanation is suggested for the infrequent occurrence in the same patient of active pulmonary tuberculosis and bronchogenic carcinoma of the squamous cell type.

The possibility should be recognized that calcified tracheobronchial lymph nodes may mark a pulmonic area particularly susceptible to the development of malignant disease.

SUMARIO

Tuberculosis Pulmonar, Bronquiectasia y Calcificación en su Relación con el Carcinoma Broncógeno

En una serie de 23 casos de carcinoma broncógeno, todos los de la forma escamocelular radicaban en el lóbulo superior ya derecho o izquierdo, todos, menos uno, estaban asociados a grandes focos calcificados en el mismo lóbulo o en los adyacentes ganglios linfáticos tráqueobronquiales, y aparte de dos, ninguno revelaba asociación con tuberculosis activa. Los tumores pulmonares de otras formas mostraron hallazgos anatómicos bastante distintos.

Ofrécese una explicación de la rara ocurrencia en el mismo sujeto de tuberculosis pulmonar activa y carcinoma broncógeno de tipo escamocelular.

Hay que reconocer la posibilidad de que los ganglios linfáticos tráqueobronquiales calcificados pueden designar una zona pulmonar en particular susceptible a la aparición de enfermedad maligna.

RESUME

Tuberculose pulmonaire, bronchiectasie et calcification en relation avec le cancer bronchiogénique

Dans une série de 23 cas de cancer bronchiogénique, toutes les cellules du type squameux ont été trouvées dans le lobe supérieur droit ou gauche. Dans tous les cas, sauf un, on a observé l'association de larges foyers calcifiés dans le même lobe ou dans les ganglions lymphatiques trachéobronchiques adjacents et tous, sauf deux, n'étaient pas associés à une affection tuberculeuse en activité. D'autres types de tumeurs pulmonaires présentaient des caractères anatomiques entièrement différents.

Une explication est offerte pour interpréter l'occurrence peu fréquente, chez un même malade, d'une tuberculose pulmonaire évolutive et d'un cancer bronchiogénique de type squameux. Il y a lieu d'envisager la possibilité que la présence de ganglions lymphatiques calcifiés marque un territoire pulmonaire particulièrement favorable au dévelopment d'une tumeur maligne.

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ADRENOCORTICAL FUNCTION IN PULMONARY TUBERCULOSIS[1,2]

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There is a striking similarity between many of the outstanding symptoms of pulmonary tuberculosis and adrenocortical insufficiency. The present study was therefore undertaken to evaluate adrenocortical function in a group of patients with pulmonary tuberculosis and to determine if adrenocortical function has any role in the determination of prognosis.

MATERIALS AND METHODS

The 50 patients included in this study were unselected cases of pulmonary tuberculosis hospitalized at Eagleville Sanatorium, Eagleville, Pennsylvania. The tests used to assay adrenocortical function were the eosinophile response to epinephrine or surgery and the inhibition of skin diffusion of a hyaluronidase-fluorescein wheal. Activation of the adrenal cortex was obtained by 0.3 cc. of 1:1,000 epinephrine subcutaneously in 46 patients. In 4 patients the alarming stimulus was thoracoplasty. Roche, Hills, and Thorn (1) have shown that surgery is an effective alarming stimulus for the adrenal gland.

Eosinophile response: The eosinophile responses were measured as suggested by Thorn (2) with the exception that the phloxine-propylene glycol diluent (3) was used. The counts were performed, employing the Levy chamber with the Fuchs-Rosenthal double ruling. A fall of at least 50 per cent in total circulating eosinophils four hours after epinephrine or surgery was considered to denote normal adrenocortical function. In those cases in which the fasting eosinophile level was below 63 per cu. mm., this test was not considered to be valid as an index of adrenocortical function. This value was chosen since it represents approximately 10 cells per counting chamber. If less than this number are present per chamber, it is believed that the percentage error is too great. Thus, an eosinopenic response to epinephrine might occur in the presence of inadequate adrenocortical function purely on the basis of an error inherent in the technique. The presence of a low fasting eosinophile count does not militate against the diagnosis of adrenocortical insufficiency.

Disappearance of hyaluronidase-fluorescein wheal: Failure to inhibit significantly the disappearance rate of the hyaluronidase-fluorescein wheal four hours after epinephrine or surgery was used to denote inadequate adrenocortical function. As discussed in a previous work (4), 1:25,000 fluorescein and hyaluronidase[3], 150 turbidity reducing units per cc., are used. From 0.01 to 0.03 cc. of the enzyme are added to the dye in a tuberculin syringe to produce a volume of 0.1 cc., which is injected into the volar surface of the forearm. The end-point for the test is noted when the presence of the dye in the skin cannot be appreciated. The test was performed in the fasting state and four hours after the subcutaneous injection of 0.3 cc. of 1:1,000 epinephrine.

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[1] From the Department of Medicine, Temple University Hospital, Philadelphia, and the Eagleville Sanatorium, Eagleville, Pennsylvania.

[2] An abstract of a thesis submitted by Dr. Finestone to the Faculty of the School of Medicine of Temple University in partial fulfillment of the requirements for the degree of Master of Science in Internal Medicine.

[3] Hydase was supplied by Dr. George E. Farrar, Jr. of Wyeth, Inc., Philadelphia, Pennsylvania.

Surgery: In 4 patients adrenocortical activation was obtained by surgery. Patients with normal adrenocortical function have an initial disappearance time of less than seven minutes with prolongation after epinephrine or the stress of surgery to sixteen minutes or more.

Criteria for clinical evaluation: In the evaluation of these cases, toxicity, chronicity, and clinical course at the time adrenocortical function was measured were the factors taken into consideration. *Toxicity* was graded from 0 to 3 on the following criteria. Grade 3 patients were those who were losing weight or were underweight, afebrile, and were discharging tubercle bacilli in the sputum. Thus, a value of 1 in the toxicity scale was given to each one of these factors. A patient with zero toxicity had sputum negative for tubercle bacilli, was afebrile, and was at normal weight or gaining weight. The presence of any single one of these factors would give the patient, in the classification used, grade 1 toxicity.

Chronicity was also graded. Those patients who were hospitalized for the first time for tuberculosis and who had symptoms for less than a year at the time adrenocortical function was measured were not considered chronic. If the patient had symptoms or was hospitalized for more than two years, grade 2 chronicity was present. Grade 3 patients were those who were hospitalized more than once for tuberculosis or who were hospitalized for more than three years at the time adrenocortical function was measured.

Based upon a comparison, both clinically and roentgenographically, at the time of admission to the sanatorium and at the time adrenocortical function was measured, the patients were divided into those who were improving, those whose disease was progressing, and those whose course was stationary.

The classification of the pulmonary lesion was that of the National Tuberculosis Association (5).

RESULTS

All of the 50 patients were white; 24 were males and 26 were females. Most of the patients (88 per cent) were between the ages of 20 and 50 years.

In regard to toxicity, only 3 of the total series showed no evidence of toxicity at the time adrenocortical function was measured. The majority of the patients tested (23 patients) showed grade 1 toxicity, 9 had grade 2 toxicity, and 15 had grade 3 toxicity.

As is probably true of most sanatorium populations, 21 of the total patients were either hospitalized more than once for pulmonary tuberculosis or were in the sanatorium over three years at the time adrenocortical function was studied (grade 3 chronicity). The next largest group (18 patients) had symptoms or were hospitalized for less than one year at the time the tests were performed (grade 0 chronicity). There were 4 patients who showed grade 1 chronicity at the time of testing and 7 with grade 2 chronicity. As can be anticipated from the statistics on chronicity of these patients, the majority of them showed a stationary clinical course (21 patients). Nevertheless, the number of patients in the group improving (17 patients) and in the group going downhill (12 patients) appears to be sufficient for a fair sampling.

The basic clinical data, including age, sex, pathology, classification according to the National Tuberculosis Association, toxicity, chronicity, clinical states, and treatment, are summarized elsewhere (6).

In case 1, which is also discussed in detail elsewhere (6), adequate histologic studies of the adrenal gland were available.

Briefly, this patient was a 66-year-old male with far advanced pulmonary tuberculosis who presented the clinical picture of Addison's disease. This was substantiated by an inadequate eosinophile response (fall of 29 per cent) after epinephrine and failure to inhibit the hyaluronidase-fluorescein wheal after epinephrine. Plasma chlorides were also low (338 gm. per 100 cc.). The patient was treated with parenteral sodium chloride, desoxycorticostrone acetate, and aqueous adrenal cortical extract. Clinically, the patient improved somewhat, but unfortunately died shortly after treatment due to the rupture of an abdominal aortic aneurism into the duodenum. Histologic study of the tissue from the autopsy of this patient revealed tuberculosis involving the lungs, liver, spleen, kidneys, and epididymus and bone (second lumber vertebra). Despite the clinical evidence of adrenocortical insufficency, histologic study of the adrenal sections showed only occasional cortical nodular hyperplasia with lipoid alteration but no tubercles.

For the purpose of simplicity in correlation of the results, the eosinophile response and the inhibition of the hyaluronidase-fluorescein wheal after epinephrine or surgery will be considered separately.

TABLE 1

Relation of Toxicity to Eosinophile Response to Epinephrine or Surgery

TOXICITY	TOTAL NUMBER OF PATIENTS	NUMBER OF PATIENTS WITH NORMAL RESPONSE	NUMBER OF PATIENTS WITH ABNORMAL RESPONSE	NUMBER OF PATIENTS FASTING EOSINOPHILE COUNTS OF LESS THAN 63 PER CU. MM.
0	3	0	2	1
1	23	8	11	4
2	9	2	3	4
3	15	6	7	2
Totals.....	50	16	23	11

Eosinophile response: A total of 16 patients showed a normal eosinophile response to epinephrine or surgery. An inadequate eosinophile response to epinephrine or surgery was found in 23 patients. It is of interest to note that, of the 4 patients in whom surgery was used to activate the adrenal cortex, 3 showed an adequate eosinopenic response while in one the fasting eosinophile content was 56 per cu. mm., which is too low for a significant evaluation of the test. Eleven patients had fasting eosinophile counts below 63 per cu. mm. and therefore the eosinopenic response cannot be considered significant.

An effort was made to relate the significant eosinophile responses to the clinical state of the patient. In table 1 there is a correlation of the grade of toxicity with the adequacy of the eosinophile response to epinephrine or surgery. It can be seen from this table that adrenocortical function, as measured by this eosinopenic test, does not appear to be related to the grade of toxicity. For example, there were 15 patients with grade 3 toxicity at the time adrenocortical function was measured. Of the group, 6 patients had the normal eosinophile response, 7 patients had an inadequate response, and in 2 patients the fasting eosinophile level was too low to make the test satisfactory. Moreover, of the 23 patients with grade 1 toxicity, 11 patients had an inadequate eosinophile response.

The relationship of the eosinophile response and chronicity is considered in table 2. It is of interest to note that, of the 23 cases with an inadequate response, 11 were in the early cases. However, 8 of the grade 3 chronic cases showed an inadequate response. As far as the clinical status of the patients was concerned, again there appeared to be little relationship between the adequacy of the eosinophile response and this factor. As an example, 6 of the 12 patients whose disease was progressing had normal eosinopenic responses, while 11 of the 17 who were improving had inadequate eosinophile responses (table 3).

TABLE 2

Relation of Chronicity to Eosinophile Response to Epinephrine or Surgery

CHRONICITY	TOTAL NUMBER OF PATIENTS	NUMBER OF PATIENTS WITH NORMAL RESPONSE	NUMBER OF PATIENTS WITH ABNORMAL RESPONSE	NUMBER OF PATIENTS WITH FASTING EOSINOPHILE COUNTS OF LESS THAN 63 PER CU. MM.
0	18	3	11	4
1	4	1	3	0
2	7	5	1	1
3	21	7	8	6
Totals	50	16	23	11

TABLE 3

Relation of Clinical Course to Eosinophile Response to Epinephrine or Surgery

CLINICAL COURSE	TOTAL NUMBER OF PATIENTS	NUMBER OF PATIENTS WITH NORMAL RESPONSE	NUMBER OF PATIENTS WITH ABNORMAL RESPONSE	NUMBER OF PATIENTS WITH FASTING EOSINOPHILE COUNTS OF LESS THAN 63 PER CU. MM.
Stationary	21	5	8	8
Progressing	12	6	4	2
Improving	17	5	11	1
Totals	50	16	23	11

Hyaluronidase-fluorescein wheal: If the initial disappearance time for the hyaluronidase-fluorescein wheal was less than seven minutes and prolongation after epinephrine or surgery was to sixteen minutes or more, normal adrenocortical function was considered to be present. There was a total of 9 patients in this group with normal values. Normal eosinophile responses were also present in 4 of these patients. However, 2 patients had fasting eosinophile levels below 63 and thus the eosinophile test of adrenocortical function was not valid. There were 3 patients who had a normal response as measured by the skin test, but showed an inadequate eosinopenic response. However, one patient had a 46 per cent fall in eosinophile count four hours after epinephrine, and this response can be considered borderline.

The next group of patients as measured by their response to the hyaluronidase-

fluorescein skin test were those with an initial reading of more than seven minutes but, after activation of the adrenal cortex, the disappearance time was prolonged to at least two times the initial reading. There was a total of 7 such responses. It is believed that this represents adequate adrenocortical responsiveness. It is possible that the prolonged initial reading may indicate some degree of hyperadrenocorticism. Adequate eosinopenic responses occurred in 4 patients, while 2 patients had fasting eosinophile counts below 63 per cu. mm. Only one of these 7 patients had an inadequate eosinophile response to epinephrine. Thus, a good degree of correlation exists between the two tests in this group.

The third type of response as measured by the skin test occurred in those patients who showed an initial reading of less than seven minutes with a failure of prolongation of disappearance time after adrenocortical activation to sixteen minutes or more. This response would represent inadequate adrenocortical function as measured by this method. There were 12 patients in this group. The

TABLE 4

Relation of Degree of Toxicity to Response to Hyaluronidase-Fluorescein Skin Test

TOXICITY	TOTAL NUMBER OF PATIENTS	NUMBER OF PATIENTS WITH NORMAL RESPONSE	NUMBER OF PATIENTS WITH INADEQUATE RESPONSE	NUMBER OF PATIENTS WITH PROLONGED INITIAL READING BUT WITH ADEQUATE RESPONSE	NUMBER OF PATIENTS WITH PROLONGED INITIAL READING BUT WITH INADEQUATE RESPONSE
0	3	0	1	1	1
1	23	6	5	4	8
2	9	0	1	1	7
3	15	3	5	1	6
Totals.....	50	9	12	7	22

eosinophile responses were adequate in 2 of these patients (the initial eosinophile counts were below 63 per cu. mm. in 3 patients), and the two tests correlated in the other 7 cases in this small group.

In 22 patients tested with the fluorescein-hyaluronidase wheal, the initial reading was longer than seven minutes but there was no inhibition of the disappearance time after adrenocortical activation. This response may represent hyperadrenocorticism in which further stress can produce no further response. More will be said about this in the discussion. Six of these patients had adequate eosinopenic responses, while in 4 patients the initial eosinophile counts were below the critical level of 63 per cu. mm. Inadequate eosinophile responses were present in 12 of these patients.

As with the eosinophile responses, an effort was made to relate the chronicity, toxicity, and clinical course of the patient to the results with the hyaluronidase-fluorescein skin test. In table 4 the results with the skin test are compared with the degree of toxicity of the patient. As was the case with the eosinophile responses, there appeared to be little correlation between the degree of toxicity and the skin test. In table 5 a similar comparison is made of the results of the skin

test with the chronicity of the patient at the time the test was done. Again there appears to be no striking correlation between the chronicity of the disease and the results of the skin test. The clinical courses of the patients as related to the skin tests are found in table 6. In this comparison, again, no striking conclusions can be drawn. However, 10 of the 17 patients who were improving were in the group characterized by a prolonged initial skin test without inhibition of the disappearance time after adrenocortical activation.

TABLE 5

Relation of Degree of Chronicity to Response to Fluorescein-Hyaluronidase Skin Test

CHRONICITY	TOTAL NUMBER OF PATIENTS	NUMBER OF PATIENTS WITH NORMAL RESPONSE	NUMBER OF PATIENTS WITH INADEQUATE RESPONSE	NUMBER OF PATIENTS WITH PROLONGED INITIAL READING BUT WITH ADEQUATE RESPONSE	NUMBER OF PATIENTS WITH PROLONGED INITIAL READING BUT WITH INADEQUATE RESPONSE
0	18	5	2	3	8
1	4	0	2	0	2
2	7	1	3	1	2
3	21	3	5	3	10
Totals.....	50	9	12	7	22

TABLE 6

Relation of Clinical Course to Response to Fluorescein-Hyaluronidase Skin Test

CLINICAL COURSE	TOTAL NUMBER OF PATIENTS	NUMBER OF PATIENTS WITH NORMAL RESPONSE	NUMBER OF PATIENTS WITH INADEQUATE RESPONSE	NUMBER OF PATIENTS WITH PROLONGED INITIAL READING BUT WITH ADEQUATE RESPONSE	NUMBER OF PATIENTS WITH PROLONGED INITIAL READING BUT WITH INADEQUATE RESPONSE
Stationary.....	21	4	6	5	6
Progressing.....	12	2	4	0	6
Improving.....	17	3	2	2	10
Totals.....	50	9	12	7	22

DISCUSSION

There have been a few previous studies, both experimental and clinical, on the relationship of tuberculosis to adrenocortical function. In none of the previous studies, however, has the eosinophile test or hyaluronidase-fluorescein skin test been used to assay adrenocortical function. Moreover, there have been no efforts to relate impaired adrenocortical function to specific factors in the clinical course of the disease.

Webb and co-workers (7) in 1921 showed, in an experimental study with tuberculous guinea pigs, that there is an increase in the absolute and relative weights of the adrenals in the progress of tuberculosis in these animals. They also stated that a similar enlargement of the adrenals occurs in other infections such as pyo-

genic abscesses and septicemia. Histologic examinations of these adrenal glands revealed no specific changes. They state that if any change is present, however, it is that of cortical hyperplasia. These writers also noted a beneficial effect of epinephrine in asthenic tuberculous patients but base this statement entirely on clinical observations. In a more recent study (8) the Robinson-Power-Kepler test for adrenal insufficiency was performed in 32 patients with pulmonary tuberculosis who did not have clinical Addison's disease. In 13 cases the test was positive, indicating some degree of adrenal cortical insufficiency. This test was positive more frequently in patients in poor general condition. After treatment of these 13 patients with a synthetic cortical hormone, reversal of test to normal occurred in all but one. No note is made of clinical benefits after this therapy.

Another group of writers (9) states that on the basis of personal observations in a great number of cases of pulmonary tuberculosis there is a coexisting hypofunction of the adrenal cortex. There appears to be no correlation between the gravity of the pulmonary condition and the degree of dysfunction according to this study by Molli and Pallazolli. In fact, insufficiency is often encountered in the initial stages of pulmonary tuberculosis and it seems that the function of the adrenal cortex has considerable influence upon the course of pulmonary tuberculosis. Abderhalden and Abderhalden (10) studied adrenal function by means of their "protective-ferment reaction" in 302 cases of pulmonary tuberculosis. They found disturbance of adrenal cortical function in 36 per cent of mild tuberculous cases and in nearly all of the severe cases. No further details of the study were given.

Cushing (11), in the dog, and Perla (12), in the albino rat, demonstrated that removal of the hypophysis increases the susceptibility of these animals to poisons and infections. Steinbach and associates (13) extended these observations to tuberculosis in rats and showed that the resistance of the rat to experimental tuberculosis is markedly lowered following hypophysectomy. This might be secondary to adrenocortical impairment.

There have been studies on the electrolyte abnormalities found in pulmonary tuberculosis which may have some relation to adrenocortical function. In 1938 Winkler and Crankshaw (14) described a new syndrome characterized by renal salt wasting and subnormal concentrations of sodium in the serum of patients with pulmonary disease. Of the 3 patients described in detail, 2 had far advanced pulmonary tuberculosis and one had carcinoma of the lung. Besides excreting salt under conditions which would normally be associated with maximal resorption of sodium, these patients rapidly eliminated intravenously administered salt and were unable to maintain the concentration of sodium in the serum at a normal level. Adrenal cortical extract was without influence on the persistent wastage of salt in one patient. These writers state that this syndrome is not associated with recognizable anatomic disease of the adrenals or kidneys. In another study (15) low sodium concentrations were found in the serum of 55 of 114 patients with tuberculosis. Balances were calculated in a small number of these patients and evidence suggestive of wastage of salt was found. Thorn and coworkers (16) on the other hand, in their study of electrolyte changes in pulmonary

tuberculosis, found no patients with a subnormal concentration of sodium in the serum. However, 2 of their patients did continue to excrete sodium after three days on a low sodium intake. In the Thorn study (16) of patients with pulmonary disease, there was a striking decrease in serum chloride concentration which appeared to parallel the reduction in vital capacity. It was believed that the decrease in serum chloride concentrations was compensated for, in part, by an increase in serum bicarbonate concentration.

In conclusion Thorn and his co-workers stated that patients with pulmonary tuberculosis differed from those with Addison's disease in the following ways. Patients with pulmonary tuberculosis were able to maintain a normal concentration of sodium and potassium and were also able to maintain a normal total blood volume. Further, tuberculous patients were able to withstand a regimen of sodium chloride deprivation with supplementary potassium medication without manifesting the changes in body weight, blood pressure, and urinary concentration of sodium and chloride typically observed in the presence of adrenal cortical insufficiency.

Munro and associates (17) in England studied 169 patients admitted consecutively to a tuberculosis hospital. Normal serum sodium concentrations were found in 114 of these patients while 55 had low values. Of the group with low serum sodium concentrations, 21 were examined post-mortem. Only 10 of these had any evidence to suggest tuberculosis of the adrenal glands. The finding, according to the authors, suggested that the function of the adrenal gland might be disturbed without showing definite lesions on section. One case briefly mentioned above (6), in which histologic studies of the adrenal were done, appears to be an example of this situation. There was strong evidence in this case that adrenocortical insufficiency existed during life and yet careful studies of the adrenal gland sections revealed no marked abnormalities. However, lipoid alterations and nodular hyperplasia were present. Rich (18) has described a peculiar type of cortical damage associated with acute infections and has discussed its possible relation to adrenocortical insufficiency. Friedman (19) has commented on the relation of clinical adrenocortical insufficiency to the anatomic finding of adrenocortical contraction.

In a recent study (20) from Eagleville Sanatorium, the serum chloride concentration was found to be significantly lower than normal in patients with tuberculosis. The total base concentration was also significantly lower than normal in these patients. There was no significant difference in the carbon dioxide content or in the pH of the serum between the normal and tuberculous patients. It was found that the patients who experienced higher degrees of fever with greater daily fluctuations showed lower concentration of serum chloride and total base than those whose temperatures deviated less from the normal range.

Sims and his workers (21) recently published an interesting study of the asymptomatic hyponatremia of pulmonary tuberculosis. They studied the kidneys and the adrenal cortex in an effort to differentiate this syndrome from Addison's disease. These investigators measured eosinophile responses to epinephrine in six of the ten patients. They found a normal response in these patients on at least

one test. Five patients had low normal or normal values for 17-ketosteroid excretion, while one patient with fever and advanced tuberculosis had a low excretion. Post-mortem examination in six of the cases showed normal adrenals in all. The writers conclude, therefore, that the ability to excrete almost sodium-free urine on a low salt diet; to retain a large amount of intravenously administered sodium without benefit of exogenous salt-retaining hormone; to respond with eosinopenia to epinephrine; and the absence of gross or histologic evidence of adrenal cortical disease at autopsy all militate against adrenal cortical functional impairment as the cause of this asymptomatic hyponatremia. Moreover, this syndrome does not appear to be a primary renal abnormality, since the renal tubules were able to respond by increased reabsorption of sodium when desoxycorticosterone acetate was given. The authors suggest that the hyponatremia occurs as a result of a chronic reduction in cellular osmolarity. The over-all effect would be an abnormal excretion of sodium in an effort to reduce the tonicity of the extracellular fluids in conformity with the primary reduction in tonicity of the intracellular fluids.

There have been efforts to relate changes in the peripheral blood to clinical and experimental variations in tuberculosis. It has already been stated that eosinopenia and lymphopenia may be responses to adrenal cortical activation. Thus, a review of previous observations on this subject might give some more indirect evidence on adrenocortical function in tuberculosis. Muller (22) has recently published an extensive monograph on the clinical significance of the blood in tuberculosis. The author examined the bone marrow in 74 patients, all but one of whom had at least moderately extensive pulmonary tuberculosis. An increased number of eosinophils were noted in only 2 cases. However, the percentage of eosinophils was not increased in the circulating blood. A marked decrease in the eosinophils of the bone marrow was found in 35.6 per cent of the patients. This fact may account for the low fasting eosinophile levels encountered in the blood of 22 per cent of the patients in the present study. The remaining 45 of the 73 patients showed approximately normal or slightly decreased values.

It has been noted by Cunningham and associates (23) that with dissemination of tuberculosis in rabbits the monocytes are increased in both the tissues and the peripheral blood and that with the regression of the lesion they are diminished. In addition, it was also observed in this study (23) that a decrease in lymphocytes accompanies susceptibility and an increase indicates resistance. Clinical observations on the role of lymphocytes and resistance to tuberculosis have also shown that an increase in the circulating lymphocytes indicates resistance, while the lymphocytes are usually low in lack of resistance (24, 25, 26). Medlar (27) found a constant decrease in lymphocytes in active tuberculosis and an increase when the disease shows a tendency toward healing or is inactive. An explanation for this decrease in lymphocytes in active tuberculosis has recently been suggested by Brandt and his co-workers (28) who showed that intravenous tuberculin (PPD) in tuberculous rabbits produced a decrease in total lymphocytes. They suggest that in active tuberculosis there is probably a certain amount of autoinoculation with tuberculoprotein and it is possible that this is responsible for the relative lymphopenia found.

It has also been noted that eosinophils were absent or few in number in acute progressive tuberculosis (29, 30). Eosinophilia, on the other hand, is considered a prognostically favorable sign in tuberculosis (31), and Muller (22) from her own observations states that, unquestionably, eosinophilia is more commonly observed in tuberculosis patients doing well clinically than in terminal cases or severely ill patients. However, in none of these cases were direct eosinophile counts performed. Thus, it appears that, if the eosinophile and lymphocyte counts are taken as an index of adrenocortical function, spread or activity of the disease is present with increased adrenocortical function.

Recently Abelson and Moyes (32) studied the eosinophile response to ephedrine in an effort to find a screening test for cortisone substitutes. The subjects used in this study were patients with pulmonary tuberculosis in a fairly static condition, afebrile, who were either awaiting or undergoing collapse therapy. A review of the results reveals some interesting findings not commented on by the investigators. The eosinophile responses to epinephrine were studied in 22 patients. Only 7 of this group showed normal responses. The other 15 showed falls of less than 50 per cent after epinephrine. This finding, noted incidentally during the course of another investigation, gives added weight to the results of the present study.

Lurie (33) in a study on the native resistance of inbred rabbit families of varying hereditary resistance to tuberculosis found that "the fundamental variant in the disease developed by these families was the degree of localization of the infection at the portal of entry." There also appeared to be some relation between the spread of tuberculosis and the spread of india ink in the connective tissue of the skin. It was shown previously by Lurie and Zappasodi (34) that male rabbits limited the spread of particulate matter more than female rabbits. This suggested that the spread of particulate matter in the skin might be under the influence of hormones. It has been shown that estrogen restricted the spread of india ink in the skin (35) while intravenous chorionic gonadotropin enhanced the spread (36). As suggested by these previous observations, Lurie and associates studied the effect of estrogen and chorionic gonadotropin on the course of tuberculosis in highly inbred rabbits (37). It was found that, in highly inbred, sexually mature rabbits, estrogen in large doses uniformly retarded the progress of the disease at the site of intracutaneous inoculation, diminished the extent of the disease in the internal organs, and suppressed to a considerable degree its dissemination in the body. Moreover, in a smaller group of highly inbred rabbits the periodic administration of chorionic gonadotropin uniformly enhanced the progress of the disease at the site of intracutaneous inoculation, increased its disseminations in the body, and in the majority of cases aggravated its extent in the external organs. In another study (38) an effort was made to study the mechanism of this action of estrogen in tuberculosis. It was found that estrogen reduced the skin sensitivity to tuberculin of rabbits sensitized either by active disease or heat-killed tubercle bacilli. There was also less response in the skin to toxic agents in estrogen-treated animals. This indicated that estrogen merely decreased the inflammatory responsiveness of the skin. It was also found in the

study that the internal tissues were not depressed in their allergic response to tubercle bacilli or its products.

As part of Lurie's studies on the constitutional factors in resistance to tuberculosis, the effect of estrogen on the adrenals was studied (39). It was found that in estrogen-treated rabbits the absolute number of circulating lymphocytes was usually reduced. This suggested that estrogen may exert an influence on experimental tuberculosis through the adrenal gland. It was noted in this study that tuberculosis caused a hypertrophy of the adrenal cortex. On the other hand, adrenal hypertrophy did not regularly follow administration of estrogen to these animals. In tuberculous rabbits under the influence of estrogen the adrenal cortex always appeared intact, however, but in tuberculous litter mates not treated with estrogen there were often areas of necrosis, hemorrhage, and leukocytic infiltration in the adrenals. The writers concluded that there was no anatomic evidence that estrogen altered adrenocortical function but the conclusion was not based on adrenocortical function studies. In another phase of this study, it was found that hyaluronidase excited a greater spreading effect in estrogen-treated rabbits than in rabbits under the influence of gonadotropin.

A somewhat similar investigation was recently reported by Birkhaug and Berle (40), who showed that the skin of allergic tuberculous guinea pigs displayed a restricting influence on the spread of an intradermal dye. On the other hand, the skin of nonimmunized tuberculous guinea pigs showed no restriction on the spread of the dye and in this respect resembled the normals.

SUMMARY

A survey of adrenocortical function has been made in 50 unselected patients with pulmonary tuberculosis. The tests used to assay adrenocortical function were the eosinophile test of Thorn and the hyaluronidase-fluorescein skin test. In 46 patients the alarming stimulus was epinephrine, while in 4 patients the responses were measured during adrenocortical activation following thoracoplasty.

In general, there was a good degree of correlation between the tests used to assay adrenocortical function.

Normal eosinophile responses to epinephrine or surgery were present in 16 patients, while inadequate responses were present in 23 patients. In 11 patients the initial fasting eosinophile count was too low for this test to be valid. There appeared to be no definite relationship between the adequacy of the eosinophile responses and the toxicity, chronicity, or clinical state of the patient.

Nine patients had normal adrenal cortical function as measured by the hyaluronidase-fluorescein skin test, 7 patients had a delay in initial disappearance of the hyaluronidase-fluorescein wheal but showed adequate inhibition after adrenocortical activation. The latter group may represent those cases with some degree of hyperadrenocorticism. Inadequate responses with the hyaluronidase-fluorescein wheal occurred in 12 patients, while 22 patients showed a prolonged initial disappearance time of the skin wheal with inadequate inhibition of the disappearance time after adrenocortical activation. This latter group may represent

a degree of adrenocortical activity where further stress can produce no further response.

There appeared to be no definite correlation between the degree of toxicity and chronicity and the response to the hyaluronidase-fluorescein skin test.

No definite conclusions can be made about the relation of the clinical course to the results with the skin test. However, 10 of the 17 patients who were improving were in the group which showed inadequate adrenocortical responses as measured by this test.

The study demonstrates that in pulmonary tuberculosis there is an alteration of adrenocortical function but the exact nature of this change could not be elucidated.

Thus, this review suggests that adrenocortical function and pulmonary tuberculosis are related in some fashion and the various observations provide a background for appraisal of the current studies of the effect of cortisone and adrenocorticotropic hormone on tuberculosis.

SUMARIO

La Función Adrenocortical en la Tuberculosis Pulmonar

En 50 tuberculosos pulmonares tomados al azar, verificóse un estudio de la función adrenocortical. Los ensayos utilizados para valorar la función adrenocortical fuerone a eosinofilorreacción de Thorn y la cutirreacción con hialuronidasa-fluoresceína. En 46 enfermos, el estímulo excitante fué la epinefrina, en tanto que en 4, se midieron las respuestas durante la activación adrenocortical consecutiva a la toracoplastia.

Las eosinofilorreacciones a la epinefrina o la cirugía fueron normales en 16 enfermos e inadecuadas en 23. En 11, la fórmula eosinofílica inicial en ayunas fué demasiado baja para prestar validez a la prueba.

Al parecer, no había relación bien definida entre la suficiencia de las reacciones eosinofílicas y la toxicidad, cronicidad o estado clínico del sujeto.

Nueve enfermos tenían una función suprarrenocortical normal conforme a la cutirreacción con hialuronidasa-fluoresceína y en 7 se demoró la desaparición inicial de la flictena producida por la hialuronidasa-fluoresceína, pero éstos mostraron inhibición adecuada después de la activación adrenocortical. El último grupo tal vez represente los casos en que existe algún hiperadrenocorticismo. En 12 casos hubo respuestas inadecuadas a la flictena por hialuronidasa-fluoresceína, mientras que 12 mostraron prolongación del tiempo de desaparición inicial de la flictena con inhibición inadecuada del tiempo de desaparición después de la activación adrenocortical. Este último grupo acaso represente cierto grado de actividad adrenocortical en el que ningún estímulo ulterior puede evocar más respuesta.

En general, hubo considerable correlación entre las pruebas usadas para valorar la función adrenocortical.

No había aparentemente correlación bien definida entre el grado de la toxicidad y la cronicidad y la respuesta a la cutirreacción con hialuronidasa-fluoresceína.

Tampoco cabe sacar conclusiones bien definidas acerca de la relación del estado

clínico con los resultados de la cutirreacción. No obstante, 10 de los 17 pacientes en vías de mejoría correspondían al grupo con inhibición inadecuada después de la activación adrenocortical.

El estudio actual demuestra que en la tuberculosis pulmonar existe alteración de la función adrenocortical, pero no pudo dilucidar la exacta naturaleza de dicha modificación.

RESUME

La fonction cortico-surrénale dans la tuberculose pulmonaire

Une enquête sur la fonction cortico-surrénale a été faite chez 50 patients non sélectionnés atteints de tuberculose pulmonaire. Les tests utilisés pour déterminer la fonction cortico-surrénale ont été le test éosinophile de Thorn et le test cutané hyaluronidase-fluorescéine. Chez 46 malades le stimulus qui influençait la réaction était l'adrénaline, tandis que chez 4 malades, ces réactions étaient mesurées au cours de l'excitation de la fonction cortico-surrénale qui succède à la thoracoplastie. La réponse éosinophilique normale à l'adrénaline, ou à l'intervention chirurgicale, était présente chez 16 malades tandis qu'une réaction inadéquate se manifestait chez 23 malades. Chez 11 malades, la première numération des éosinophiles, pratiquée chez le sujet à jeun, avait une valeur trop basse pour que ce test soit valable.

Aucune relation définie n'est apparue entre la précision de la réaction éosinophile, la toxicité, la chronicité ou l'état clinique du malade.

Chez 9 malades la fonction cortico-surrénale, mesurée par le test cutané hyaluronidase-fluorescéine, était normale; chez 7 malades il s'est produit un retard dans l'effacement de la papule causée par le mélange hyaluronidase-fluorescéine, mais l'inhibition de la réaction se montrait adéquate à la suite de la stimulation de la fonction cortico-surrénale. Ce dernier groupe pourrait représenter les cas où existe un certain degré d'hypercorticisme. Des réactions inadéquates du test hyaluronidase-fluorescéine ont été observées dans 12 cas, et dans 22 cas il s'est produit un retard dans le début de l'effacement de la papule et la stimulation de la fonction cortico-surrénale n'a pas eu pour résultat une inhibition adéquate du temps d'effacement de la papule. Ce dernier groupe pourrait représenter un degré d'activité cortico-surrénale tel que toute nouvelle stimulation ou "stress" demeure sans influence.

En général il existait une assez bonne corrélation entre les tests utilisés pour déterminer la fonction cortico-surrénale.

Il n'a pas été constaté de corrélation définie entre le degré de virulence et de chronicité de l'affection et la réaction au test cutané hyaluronidase-fluorescéine.

Aucune conclusion définie n'a pu être faite en ce qui concerne la relation entre l'état clinique et les résultats obtenus avec le test cutané. Toutefois 10 sujets, parmi les 17 malades qui étaient en voie d'amélioration appartenaient au groupe dans lequel l'inhibition de la réaction était inadéquate après stimulation de la cortico-surrénale.

Cette étude démontre que dans la tuberculose pulmonaire il existe une altéra-

tion de la fonction cortico-surrénale, mais la nature exacte de ce phénomène n'a pu être élucidée.

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COMPLICATIONS OF ARTIFICIAL PNEUMOPERITONEUM[1,2]

A Review

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INTRODUCTION

Artificial pneumoperitoneum has long been employed in the treatment of abdominal tuberculosis, for diagnostic purposes, and in the treatment of pulmonary emphysema. It reached its universal use, however, with its introduction in the treatment of pulmonary tuberculosis by Vajda in Europe and Banyai in this country in the early nineteen thirties. Since then, especially during the last decade, it has been used on an increasing scale. Among the reasons for its popularity are the facts that it can be established and re-established without difficulty and that it is free from the serious pleural and pulmonary complications so often encountered with artificial pneumothorax. Pneumoperitoneum may be associated with a variety of complications, however, ranging from mild discomfort to sudden death and, because of the relatively infrequent occurrence of these events, they are perhaps not so well known as should be the case. It is the purpose of the present paper to consider the complications by means of a review of the literature augmented by illustrative cases observed at the Tucson, Arizona, Veterans Administration Hospital.

MINOR INITIAL COMPLICATIONS

Pain: Induction of pneumoperitoneum is quickly followed by pain. Elevation and stretching of the diaphragm stimulate nerve fibers within its substance, and the resulting pain is referred through the phrenic nerve to the neck and shoulders. Commonly, the pain is more severe on the right side for two reasons: (*1*) More pressure is exerted upon the right diaphragm because the liver, a solid organ, does not yield as readily to the pressure of the air column collecting between it and the diaphragm as does the stomach, a hollow organ, on the left side (5). (*2*) Tension may be created in the falciform ligament as the liver is separated from the diaphragm (25). Lower chest pain is caused by pull along the origin of the diaphragm on the inside of the thoracic cage and stimulation of intercostal nerves. Abdominal discomfort is due to air pressure, peritoneal irritation by the injected air, and stretching of pre-existing adhesions between diaphragm and abdominal organs. Most observers agree that the pain is transient and disappears after the first few refills and certainly does not persist longer than six weeks after induction of pneumoperitoneum (60). The pain has been described as not severe

[1] From the Tuberculosis Service, Veterans Administration Hospital, Tucson, Arizona.

[2] Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the author are the result of his own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

enough to require medication (5), a trifling discomfort (20), and seldom sufficient cause to abandon pneumoperitoneum (2). Moyer (39), on the other hand, mentioned a few patients in whom pain was severe but was relieved by elevation of the foot of the bed. In none of his 550 patients did the presence of pain lead to discontinuation of therapy. Other writers (8, 17, 42, 60) have abandoned pneumoperitoneum because of severe pain in 0.75 to 3 per cent of their patients.

Furman and Callaway (23) treated seven patients with severe pulmonary emphysema with pneumoperitoneum and abandoned it in two because of pain. However, pulmonary emphysema associated with asthma and bronchiectasis, as it often is, differs from tuberculosis. There is generally considerable basal pulmonary infection with diaphragmatic pleurisy leading to extensive adhesions between diaphragm and abdominal organs, which will cause severe pain when put under tension.

Anorexia and loss of weight: Anorexia and loss of weight follow commonly after induction of pneumoperitoneum and are the result of fullness of, and pressure in, the abdominal cavity. As an exception, Bennett (8) noted improvement in appetite and weight. Habeeb and Reiser (25) called anorexia (and nausea) a benign complication occurring during the early phase of pneumoperitoneum therapy. One-half of Mellies' (33) patients first lost weight and regained it slowly. Once a maximum rise of the diaphragm has been obtained and further large refills are given, the abdominal viscera will be displaced downward, resulting in loss of appetite and weight (18). Edwards and Logan (20), calling initial loss of weight their most striking complication, concluded it was not related to clinical status. This is true, but continued loss or gain of weight after pneumoperitoneum has been stabilized is undoubtedly governed by the activity of the underlying tuberculous lesion.

Fever and vertigo: Aslett and Jarman (3) induced pneumoperitoneum following phrenic crush in two patients in poor general condition with advanced tuberculosis, and observed considerable febrile reaction after induction and each of two refills. Daniels and Eisele (18) noted slight vertigo in six patients after the initial inflation and in three patients after refills.

CARDIAC AND PULMONARY COMPLICATIONS

Dyspnea: Pneumoperitoneum lowers the vital capacity by an average of 12.5 per cent (5), yet it rarely causes dyspnea. This symptom has usually been noted in only a few patients (8, 33) and even previously present dyspnea remains unchanged (42). Dyspnea may appear, however, when the intra-abdominal pressure becomes high enough to suspend motion of both leaves of the diaphragm (22). Anderson and Winn (1) reported the occurrence of dyspnea in 6 per cent of their patients but in only one series were so many as two-thirds of the patients dyspneic (18).

Cardiac failure: Pneumoperitoneum may cause cardiac decompensation in older people and in those with an already damaged heart. Case (13) mentions a man sixty years of age who a few moments after inflation for diagnostic purposes went into acute heart failure but recovered. Fowler (21) had a similar experience

TABLE 1

Complications of Artificial Pneumoperitoneum

Minor initial complications	Pain Anorexia and loss of weight Fever Vertigo
Cardiac and pulmonary complications	Dyspnea Cardiac decompensation Aggravation of hemoptysis Pulmonary atelectasis Basal pulmonary abscess Accidental and spontaneous pneumothorax
Gastrointestinal and abdominal complications	Nausea and vomiting Constipation and diarrhea Flatulence Hiccough Aggravation of intestinal tuberculosis Melena, rectal hemorrhage Aggravation of hemorrhoids Peritoneal effusion Tuberculous peritonitis Generalized peritonitis Peritoneal adhesions and obliterative peritonitis Intra-abdominal hemorrhage Puncture of viscera Acute appendicitis Torsion of spleen or omentum Hemorrhage into spleen Needle broken off in abdomen
Complications of abdominal wall	Subumbilical hematoma Umbilical pneumocele Diastasis of rectus muscles Hernia Scrotal pneumocele
Urinary and gynecologic complications	Urinary retention Amenorrhea, dysmenorrhea Cystocele, rectocele
Interstitial emphysema and air embolism	Subcutaneous and preperitoneal emphysema Omental emphysema Mediastinal emphysema Air embolism

with a man fifty-four years of age who was known to have coronary sclerosis. Displacement of the heart and distortion of the great vessels were considered to have been responsible. Anderson and Winn (1) reported the death of a patient who had had pre-existing heart disease, and Trimble and associates (60) aban-

doned pneumoperitoneum in two patients who showed cardiac embarrassment complicating cor pulmonale. Patients with normal hearts are unlikely to develop cardiac decompensation (5, 21) and even advanced age and mild cardiovascular disease do not contraindicate pneumoperitoneum if smaller refills are given at the regular intervals (1, 5).

Hemoptysis: Pneumoperitoneum actually caused pulmonary bleeding in a case described by Mellies (33). Upon removal of some air, hemoptysis stopped but recurred when larger refills were resumed. Mellies believed that the phenomenon resulted from a cavity which had been fixed in its position, and attempts at elevation resulted in tears of its walls. Likewise, Mitchell and co-workers (37) observed diminution in hemoptysis in seven patients following cessation of pneumoperitoneum.

Collapse of pulmonary tissue: Massive (11) and smaller areas of pulmonary atelectasis (33, 58) have been observed during pneumoperitoneum therapy caused by bronchial kinking and interference with drainage. The smaller areas cleared up promptly (33) but Iturriaga (28) reported a patient in whom atelectasis (caused by phrenic crush and pneumoperitoneum) provided an excellent medium for secondarily invading *Streptococci*, causing development of a basal lung abscess.

Pneumothorax: Accidental pneumothorax may complicate pneumoperitoneum when the subdiaphragmatic route is used for air inflation. The tip of the needle may be accidentally introduced into the pleural rather than the peritoneal cavity (5). Several such cases have occurred (5, 24). For this reason alone the paraumbilical approach is distinctly preferable.

Recently Ross and Farber (51) have called attention to spontaneous pneumothorax as a complication of pneumoperitoneum. All cases with loss of air from the abdomen were right sided. They quoted five previously reported cases (6, 33, 53, 55, 66), added three of their own, and collected another twelve from California physicians. An additional case has since been reported by Wu and Neptune (65). It has been explained that air from the peritoneal cavity passed through the diaphragmatic openings along the large structures, forced its way into the pleural space, and caused a pneumothorax (6, 53). Others (1, 33, 55, 57, 65, 66) believed that air passed through an inflammatory or congenital defect in the diaphragm from the peritoneal into the pleural cavity. Spontaneous rupture of the diaphragm may occur when there is far advanced disease and an erosive ulcerative tuberculous lesion of the diaphragm (1) or if the muscle fibers are sufficiently stretched apart to permit a small rupture of the diaphragm resulting in bleb formation on its pleural surface (44, 66).

Illustrative cases: The following cases are of special interest. Mellies' (33) patient suffered a perforation of the right diaphragm after five pneumoperitoneum treatments resulting in a spontaneous pneumothorax; she was in a critical condition for ten days but recovered. A patient observed by Yannitelli and coworkers (66) developed a partial right spontaneous pneumothorax during the course of pneumoperitoneum; following re-expansion of the lung, refills were cautiously resumed. Several months later she died rather suddenly with signs of

respiratory distress. Autopsy revealed a right tension pneumothorax and no free air space in the abdomen. On the peritoneal surface of the diaphragm was a round opening, 2 mm. in diameter, which communicated with a bleblike mass on the pleural surface. Very recently a similar nonfatal case was reported by Repa and Jacobson (44).[3] Smith (57) watched the induction of pneumoperitoneum for diagnostic purposes in a young woman. As soon as the inflation was begun the patient became dyspneic and complained of chest pain; then she collapsed and died shortly after. Autopsy revealed bilateral pneumothorax and a number of small, apparently congenital openings connecting the peritoneal with both pleural cavities.

GASTROINTESTINAL AND ABDOMINAL COMPLICATIONS

Nausea and vomiting: Excessive nausea and vomiting are rare. Fremmel (22) noted it only with high abdominal pressures. Edwards and Logan (20) saw a patient who had two violent attacks of vomiting each time after a refill. Gilmore (24) abandoned pneumoperitoneum for this reason in two patients in whom emotional factors played an important part. Two of Mellies' (33) patients vomited for twenty-four hours; in both, the left diaphragm was more elevated.

Bowel disturbances: Constipation has been reported in several patients (5). One of our patients whose bowels had always been regular prior to pneumoperitoneum became constipated for two to three days after each refill. Hiccough of short duration (22, 54), persistent diarrhea (5), and considerable flatulence (8, 18) have occurred. Pre-existing symptoms of intestinal tuberculosis may be aggravated to such a degree that pneumoperitoneum will have to be abandoned (19, 58). Melena following pneumoperitoneum (47) and a brisk rectal hemorrhage after a refill have been described (20). In the latter case the blood was bright red and its source could not be found. A man treated with pneumoperitoneum for severe pulmonary emphysema by Furman and Callaway (23) complained of severe epigastric pain of twelve hours' duration accompanied by passage of a bloody stool. The pain was considerably relieved by removal of air, recurred later on, and subsided the following day; refills were later resumed without further incident. The writers believed that pain and bloody stool were caused by trapping of air beneath a section of mesentery. In another of their patients, hemorrhoids were severely aggravated and treatment had to be abandoned.

Peritoneal effusions: Sterile peritoneal effusions occurring during pneumoperitoneum therapy are frequently mentioned (1, 2, 3, 8, 18, 19, 20, 21, 25, 37, 39, 53, 60). In most cases the fluid is small in amount, asymptomatic, and does not interfere with further refills. Anderson and Winn (1) considered it a warning not to increase intra-abdominal pressures to too high a level. In some patients, fever, malaise, abdominal soreness, nausea, and vomiting may accompany the appearance of the fluid. If it increases, aspiration and cessation of pneumoperitoneum are indicated. Fowler (21) remarked that its deleterious effects depend more on the causative lesion than on the effusion itself. After study of autopsy

[3] Since receipt of this paper, two more nonfatal cases of right-sided spontaneous pneumothorax caused by rupture of the diaphragm have been reported.

findings on patients who had been treated with pneumoperitoneum, Trimble, Eaton, and Moore (61) concluded that a large peritoneal effusion complicating pneumoperitoneum is strongly suggestive of tuberculous peritonitis. Others (2, 53) have concurred in this opinion. In the large reported series the frequency of peritoneal effusion was approximately 1 to 3 per cent (8, 25, 37, 39, 53, 60); only Anderson and Winn (1) reported a figure as high as 8 per cent.

At this hospital one case of excessive fluid formation was observed; tuberculous peritonitis was a strong possibility but could not be proved.

G. F. M., a 53-year-old white male, re-entered the hospital January 30, 1948, complaining of moderate fever and increased productive cough. His sputum was positive for tubercle bacilli, and roentgenogram of the chest showed infiltration of the entire right lung field with recent progression and several fibrocalcareous deposits in the left lung field. His daily temperatures which ranged to almost 102° F. dropped to 100° F. in mid-February. Pneumoperitoneum was induced February 20, 1948. Late in April 1948, abdominal fluid appeared, causing dyspnea. Pneumoperitoneum was discontinued after twelve refills. In May and June, 1948, two abdominal paracenteses were done, and 2,800 and 1,000 cc., respectively, of slightly cloudy, straw-colored fluid were obtained. Specimens of the fluid were negative for tubercle bacilli on smear and culture. Thereafter no appreciable amounts of fluid formed. The patient continued on his downhill course despite streptomycin therapy and died in October, 1948. An autopsy could not be obtained.

Peritonitis: Tuberculous peritonitis, a very serious complication, occurs in approximately 1 per cent of patients treated with pneumoperitoneum for pulmonary tuberculosis (1). Crow and Whelchel (17) noted five cases among their 546 patients (0.9 per cent); one recovered and four died. Trimble and associates (60) encountered one case and discontinued therapy. It has been said that in most cases the tuberculous peritonitis probably preceded the pneumoperitoneum and that the latter merely served to reveal its presence (1, 5, 25).

Case (13) reported a fatality from a generalized peritonitis after diagnostic pneumoperitoneum in a patient with carcinoma. Rilance and Warring (46) observed the development of an acute peritonitis following rupture of a chronic pelvic abscess. Mitchell and associates (37) observed two cases of mixed-infection peritonitis.

Peritoneal adhesions: Peritoneal adhesions are commonly seen during pneumoperitoneum treatment. Anderson and Winn (1) noted adhesions in 71 per cent of their patients. Generally, the adhesions are asymptomatic but in an occasional case may cause severe pain from tension or prevent a satisfactory rise of the diaphragm and force abandonment of therapy. A trapped loop of bowel may lead to intestinal obstruction and a torn adhesion can give rise to intraperitoneal hemorrhage. In rare instances, obliterative peritonitis will occur and make further refills impossible (1, 8, 33, 60, 62). Severe adhesion formation prevented reinstitution of pneumoperitoneum in two instances reported by Corrigan (16). The writer has observed the following instance of this type of complication.

F. W., a white male, age 27, gave a history of pulmonary tuberculosis with sputum positive for tubercle bacilli since 1943. Pneumoperitoneum was induced in 1944 and reinforced with left and right phrenic crushes in 1944 and 1945, respectively. After sputum

"conversion to negative" he was treated as an outpatient. In October 1947, he was admitted here because of fever and ascites. One sputum was found positive for tubercle bacilli at the time. Abdominal aspiration produced 600 cc. of turbid fluid which subsequently was found to be sterile. Fluoroscopy revealed no further free fluid and pneumoperitoneum was continued. The patient left the hospital in January 1948. A private physician removed 1,000 cc. of air from his abdomen and started a course of streptomycin. He was readmitted on February 20, 1948, with the chief complaints of nausea, vomiting, and abdominal pain of forty hours' duration. Prior to admission his vomitus assumed fecal character.

A diagnosis of intestinal obstruction was made and immediate laparotomy disclosed the following findings. The entire abdomen was the site of soft recent adhesions. A large fibrous mass extended from the epigastric area down below the umbilicus. This apron-like structure to which the small bowel was adherent had two leaves and contained some serous fluid. One small bowel loop was completely obstructed by a band adhesion which encircled it. A knot of small bowel had formed near the terminal ileum as a result of numerous dense adhesions. The stomach was likewise bound down by numerous soft adhesions, and a considerable amount of free fluid was found in the abdominal cavity. The stomach and intestines were freed, mainly by blunt dissection, and the abdomen was closed. The patient made an uneventful recovery and to date has not had a recurrence of abdominal symptoms. The pathologic report on removed adhesion tissue was: *Fibrinopurulent debris, fragments of infarcted fibrous and fatty connective tissue with luxuriant granulation tissue, and chronic inflammation.*

Intraperitoneal hemorrhage: In 1947 Cornwall and Patridge (15) reported a death from massive intraperitoneal hemorrhage approximately six hours after a pneumoperitoneum refill. Autopsy revealed a punctured small vein in the greater omentum as the bleeding point. Larkin and Phillips (30) observed a nonfatal case; laparotomy disclosed a large amount of bloody fluid in the abdominal cavity but no bleeding point. They assumed that either a punctured abdominal vessel or a ruptured vascular adhesion caused the hemorrhage.

Puncture of viscera: Puncture of abdominal viscera might seem to be an important hazard of pneumoperitoneum therapy but only a few such instances have been recorded. The accident may occur more frequently but remain unrecognized (53). Case (13) reported three punctures of the intestines, one each of the urinary bladder and a hydroureter without ill effects, and a death from oxygen injection into the spleen. Fatal air embolism has occurred following air injection into the liver parenchyma (27, 37, 43, 53). In Clifford-Jones' and McDonald's (14) experience an intestinal perforation remained without sequelae, and pneumoperitoneum was resumed a few weeks after the incident. Puncture of the stomach occurred once in this hospital.

H. C. M., a poorly nourished white male, age 57, had pneumoperitoneum induced on June 24, 1946 for treatment of bilateral pulmonary tuberculosis. At the time of the second refill on July 1, 1946, no initial reading was obtained but 2 cc. of yellowish fluid having the characteristic odor of gastric juice were drawn into the syringe. There were no untoward effects and pneumoperitoneum was continued one week later.

Appendicitis: Rilance and Warring (46) in 1944 reported that acute appendicitis occurred eleven times more frequently among their patients with pneumoperitoneum (6.9 per cent) than among the remainder of their sanatorium population (0.64 per cent). Knoepp (29) found a ratio of 7:1 and Schaff and Bornstein (52)

of 6:1 while others noted no increase (1, 37, 39) or encountered no cases at all (53, 60). Acute appendicitis seems to occur slightly more often in patients with pneumoperitoneum than in the other hospitalized tuberculous patients. More important than its frequency, however, is the fact that, in the presence of free air in the abdominal cavity, no walling off of the diseased appendix takes place and a generalized peritonitis will rapidly spread (29). Knoepp (29), after observing two cases, strongly advocated early surgical exploration of any suspected case of acute appendicitis in the presence of artificial pneumoperitoneum. One of the writer's cases emphasizes this point.

J. C. Y., a 28-year-old male with far advanced pulmonary tuberculosis, had pneumoperitoneum induced in January 1949. During the afternoon of September 22, 1949, he complained of severe epigastric pain followed by loose stools and pain across the lower abdomen. These symptoms were temporarily relieved by medication. The next morning he again complained of pain in the lower abdomen. Mild rebound tenderness and pain referred to the right lower quadrant were present; rectal examination was negative. The total leukocyte count was 17,000 per cu. mm. with 84 per cent polymorphonuclear cells. Appendectomy was performed at 4 p.m., September 23, 1949. There was free pus in the abdominal cavity, and the appendix had a 3 mm. perforation near its tip; there was no noticeable attempt at walling off the appendiceal area. The patient recovered after a stormy postoperative course.

Miscellaneous intra-abdominal complications: The following conditions have been reported once: torsion of the spleen (36), hemorrhage into the spleen (60), and a needle broken off in the abdomen (37). Each case required surgery. Torsion of the omentum caused pneumoperitoneum to be abandoned in one patient (60).

COMPLICATIONS OF THE ABDOMINAL WALL

Subumbilical hematoma, umbilical pneumocele, and diastasis of the muscles have on rare occasions been observed (5, 47). Aggravation of pre-existing, or development of new, hernias (either inguinal, umbilical, femoral, or incisional) have been commented on by many writers. A good many of the hernias will be adequately controlled by a truss and lowering of the intra-abdominal pressure (1, 5, 17, 18, 25, 46) while some will force discontinuation of pneumoperitoneum (8, 11, 25, 60). It has been suggested that all patients slated for pneumoperitoneum be screened for relaxed rings (17) or hernias (40) and, when indicated, be fitted with a truss or, whenever feasible, have the hernia repaired (23) prior to induction of pneumoperitoneum. In this manner this treatment is not necessarily contraindicated in ruptured patients (17). In the large reported series the frequency of hernias during pneumoperitoneum therapy was 1.5 to 3 per cent (8, 17, 37). Only two patients are mentioned in the literature whose hernias were repaired during pneumoperitoneum treatment which was resumed after a short postoperative period (32, 40). The writer has followed this course in one of the outpatients. Incarceration of a (femoral) hernia was once encountered (37). Rilance and Warring (45) reported the filling of an already existing small inguinal sac with fluid. The hernia could not be reduced until a small amount of fluid had been aspirated.

Scrotal pneumocele as a complication of pneumoperitoneum has been described

eight times (5, 9, 34, 38, 40, 49). Twice it occurred after the initial inflation and in the remainder after a varying number of refills. The pneumocele is due to a persistent patent processus vaginalis connecting the peritoneal cavity with the scrotal tunica vaginalis. Normally this passageway becomes obliterated after descent of the testicle into the scrotum. Where pneumocele did not occur with the initial inflation, no open passage may have existed originally but the air forced the weakly adherent walls of the processus vaginalis apart (49). Pain subsided in all cases within twenty-four hours, and scrotal swelling disappeared usually in four to six days. In no case was there a recurrence with further refills, even if no pressure pad was applied over the inguinal ring. The irritating effect of the air may have brought about final closure of the processus vaginalis (38). An additional case of this complication has been observed by the writer.

D. R. E. McB., a 31-year-old white male, had his initial pneumoperitoneum inflation on March 21, 1949; 500 cc. of air were injected, and the final reading was plus 12. Two hours later he noted soreness of the left side of the scrotum and across the pubic arch. Five hours after the procedure the scrotum had reached the size of a small grapefruit and was extremely tense. Tenderness disappeared within twenty-four hours but it took nine days for the scrotum to return to its normal size. Pneumoperitoneum was reinduced two months later without recurrence of pneumocele.

URINARY AND GYNECOLOGICAL COMPLICATIONS

Urinary retention during pneumoperitoneum therapy has been observed in two cases (10); in both, prolonged catheterization was necessary until normal function returned after ten days and three months, respectively.

Amenorrhea (33) and slight degrees of dysmenorrhea (20) have been reported in connection with pneumoperitoneum. Treatment should not be initiated during menstruation (5) because severe uterine cramps might follow but refills may be given at any time of the menstrual cycle (5, 20). Pelvic inflammatory disease can force cessation of treatment (60). Cystoceles and rectoceles may be aggravated or caused by pneumoperitoneum. Insertion of a pessary and decreased frequence or size of refills will give sufficient relief to permit continuation of treatment (50).

INTERSTITIAL EMPHYSEMA AND AIR EMBOLISM

Subcutaneous emphysema is relatively frequent, of short duration, and of no importance. Similarly, preperitoneal and omental emphysema (5) and abdominal pneumocele (64) have no clinical significance. Mediastinal emphysema is a rare complication, fourteen cases having been reported (1, 7, 39, 53, 56). When air is given by the subdiaphragmatic route, the sudden increase of pressure in that area may be the cause or air may make its way from the peritoneal cavity into the mediastinum through a diaphragmatic hiatus along the large structures passing through the diaphragm (5). Symptoms may be moderately severe but usually disappear in one to four days. The following case report is an illustration of this type of complication.

M. E. G., a 29-year-old white male, had a pneumoperitoneum since March 5, 1948, without discomfort. A refill of 750 cc. was given April 2, 1948. The patient had no complaints

but four hours later, immediately after a hard coughing spell, he noted severe substernal pain, palpitations, and difficulty and pain on swallowing. Half an hour later, subcutaneous emphysema appeared on the anterior aspect of the neck. A tympanitic percussion note was elicited lateral to both sternal borders. Roentgenographic examination revealed air beneath the sternum and in the deep fascial planes of the neck. The next day symptoms were improved but some substernal pain and soreness persisted. Five hundred cubic centimeters of air were removed from the abdomen. By April 7, 1948 all symptoms and the subcutaneous emphysema of the neck had disappeared. Pneumoperitoneum was continued with smaller refills at shorter intervals without recurrence.

Air embolism: The most serious complication of pneumoperitoneum is air embolism. Fifteen fatal cases have been reported to date (4, 13, 26, 27, 31, 37, 43, 48, 53, 54, 59, 60, 62, 63).[4] Actually, more have occurred in various institutions but have not been published, and for this reason it is difficult to establish its true frequency. Some were caused by air injection into the substance of the liver (27, 37, 43, 53), into a splenic vein (26, 62), and into the iliac artery (59). In the remainder of the cases, the point of entry into the circulation remained obscure. Nonfatal air embolism has occurred in greater numbers (2, 12, 22, 33, 43, 45, 53), sometimes confusingly called "nervous accident" (41). It is more apt to occur on induction but may happen with refills in a well-established pneumoperitoneum. The writer encountered one nonfatal case during a refill in approximately 14,000 air inflations.

Three deaths during pneumoperitoneum treatment have been described (3, 35) in which the cause could not be determined clinically and no autopsies were performed. Delayed air embolism may well have been responsible, similar to the case reported by Warring and Thomas (63).

SUMMARY

Artificial pneumoperitoneum is in extensive use today, mainly in the treatment of pulmonary tuberculosis. Contrary to general belief, many more than forty different complications may be encountered during its course. These have been reviewed from the literature. Several new cases have been added.

Minor complications, such as initial pain and loss of weight, hematoma of the abdominal wall, and various forms of interstitial emphysema, are generally of little clinical significance. Of these, loss of weight occurs in approximately one-half of all patients receiving pneumoperitoneum. Severe initial pain will render further refills impossible in 0.75 to 3 per cent.

Cardiac decompensation and various surgical abdominal conditions may force discontinuation of pneumoperitoneum, depending on their severity. The same holds true for hernias which occur in 1.5 to 3 per cent of the patients treated, peritoneal effusions which occur in 1 to 3 per cent, and peritoneal adhesions which are present with a frequency of 71 per cent.

Increased hemoptysis, aggravation of intestinal tuberculosis, and various types of peritonitis require cessation of therapy. Of these, tuberculous peritonitis has been noted in 1 per cent of all patients receiving pneumoperitoneum.

[4] Another death has been recently reported since receipt of this paper.

Immediate or delayed death may be caused by intra-abdominal hemorrhage (one fatal case on record), spontaneous pneumothorax (2 deaths reported), and air embolism (15 fatalities described).

From the above it appears that, of all patients who receive artificial pneumoperitoneum, approximately 5 to 6 per cent will develop complications serious enough to make continuation of therapy impossible.

SUMARIO

Complicaciones del Neumoperitoneo Terapéutico

El neumoperitoneo articial tiene amplia aplicación hoy día, sobre todo en el tratamiento de la tuberculosis pulmonar. En contraposición a la creencia general, pueden encontrarse más de 40 distintas complicaciones durante su evolución. La literatura relativa a las mismas ha sido repasada y ahora se agregan varios casos nuevos.

Las complicaciones menores, tales como dolor y pérdida de peso al principio, hematoma de la pared abdominal y varias formas de enfisema intersticial, poseen en general poca importancia clínica. De ellas, la pérdida de peso afecta aproximadamente a la mitad de todos los enfermos neumoperitoneizados. El intenso dolor inicial vedará nuevas reinsuflaciones en 0.75 a 3 por ciento.

La descompensación cardíaca y varios estados quirúrgicos del abdomen pueden imponer, conforme a su gravedad, el abandono del neumoperitoneo. Lo mismo reza con las hernias que ocurren en 1.5 a 3 por ciento de los casos, los derrames peritoneales con una incidencia de 1 a 3 por ciento y las adherencias peritoneales con una frecuencia de 71 por ciento.

El aumento de la hemoptisis, la agravación de la tuberculosis intestinal y varias formas de peritonitis exigen el cese de esa terapéutica. De éstas, se ha observado peritonitis tuberculosa en 1 por ciento de todos los enfermos tratados con neumoperitoneo.

La muerte inmediata o tardía puede ser ocasionada por hemorragia intra-abdominal (hay un caso letal comunicado), neumotórax espontáneo (2 muertes comunicadas) y embolia gaseosa (15 muertes comunicadas).

A juzgar por lo anterior, parece que de todos los enfermos que reciben el neumoperitoneo terapéutico, aproximadamente 5 a 6 por ciento manifestarán complicaciones de gravedad suficiente para impedir la continuación de la terapéutica.

RESUME

Complications du pneumopéritoine artificiel

Il est fait actuellement un usage extensif du pneumopéritoine artificiel, principalement dans le traitement de la tuberculose pulmonaire. A l'encontre de la croyance générale, beaucoup plus de 40 complications différentes peuvent être observées pendant qu'il est en cours. Ces complications ont été passées en revue. Plusieurs nouveaux cas ont été ajoutés.

Les complications mineures, telles que la douleur initiale et la perte de poids,

l'hématome de la paroi abdominale et diverses formes d'enphysème interstitiel n'ont en général qu'une importance clinique réduite. Parmi ces complications la perte de poids se manifeste dans environ la moitié des cas traités par le pneumopéritoine. La douleur initiale sévère rendra toute nouvelle réinsufflation impossible dans 0,75 à 3% des cas.

La décompensation cardiaque et diverses conditions abdominales d'ordre chirurgical peuvent, selon leur gravité, nécessiter l'interruption du pneumopéritoine. Ceci est également vrai pour les hernies, qui se produisent dans 1,5 à 3% des cas, les épanchements péritonéaux, dont l'incidence est de 1 à 3%, et les adhérences péritonéales dont la fréquence est de 71%.

L'augmentation des hémoptysies, l'aggravation de la tuberculose intestinale et divers types de péritonite réclament l'interruption du traitement. La péritonite tuberculeuse a été observée chez 1% de la totalité des malades soumis au pneumopéritoine.

La mort immédiate ou retardée peut être causée par l'hémorragie intra-abdominale (un cas mortel est enregistré), le pneumothorax spontané (2 cas de mort sont signalés) ou l'embolie gaseuse (15 décès sont décrits).

De ce qui précède il apparaît que, parmi les malades soumis au pneumopéritoine artificiel il y en a 5 à 6% chez lesquels ce développent des complications suffisamment sérieuses pour prévenir la continuation de la thérapie.

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EXPERIMENTAL STUDIES ON THE TOXICITY OF AMITHIOZONE IN DOGS[1]

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Amithiozone (4-acetyl aminobenzaldehyde thiosemicarbazone) is a relatively new antituberculous agent developed by Domagk, Behnisch, Mietzsch, and Schmidt (1). It has been investigated extensively in Germany where it has been found to be effective in treating tuberculosis of the mucous membranes and skin (2, 3, 4), exudative forms of pulmonary tuberculosis (2, 5), and in other types of tuberculosis in which it can be applied directly to the foci of infection (2). Reports of relatively high toxicity (2, 6, 7), however, have limited its use as a therapeutic agent. Most of the toxicity data in this country has been accumulated from patients with active tuberculosis. Under these circumstances, it is frequently difficult to differentiate symptoms due to drug toxicity alone from symptoms due to the underlying disease. The maximum tolerated dosage of amithiozone has not been well defined. Approximately 2 mg. (8), rarely up to 10 mg. (9), per kg. of body weight per day have been used clinically, yet doses of more than 35 mg. per kg. (10) have been necessary to produce maximal inhibition of tubercles in mice.

The present report is concerned with a study of the toxicity of amithiozone[3] in dogs, with particular reference to the liver, kidneys, and hematopoietic system. An attempt has been made to evaluate the severity and frequency of toxic reactions related to increasing dosage of the drug. This approach may suggest a method of therapy which will allow maximum therapeutic benefit with minimal toxic reactions in selected patients in whom amithiozone is indicated for the treatment of tuberculosis.

MATERIALS AND METHODS

Twenty-two dogs which ranged in weight from 14 to 23 kg were used. The animals were separated into 2 groups. *Group I* consisted of 12 dogs which were given large doses of amithiozone and studied for acute toxicity. *Group II* consisted of 10 dogs which were given smaller doses and were studied for chronic toxicity over a period of six months.

Group I: Seven dogs (*Group IA*) received an oral dose of 100 mg. of amithiozone per kg. daily for five days, and 5 (*Group IB*) received 50 mg. per kg. per day for five days. After this period of time all of the dogs in *Group I* were sacrificed. In addition to gross examination of all organs, microscopic examinations were made of the heart, lungs, liver, brain, spleen, pancreas, kidneys, and bone marrow[4].

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[2] Deceased.

[3] Supplied as Tibione through the courtesy of Schenley Laboratories.

[4] In addition to the authors' observations, all specimens were reviewed by Dr. L. S. Smith, Department of Pathology, Baylor University College of Medicine.

Group II: Ten dogs were placed on a schedule of 10 mg. of amithiozone per kg. daily for three months and 20 mg. per kg. daily for an additional two months. At the end of this time, 2 had died and 3 of the animals were sacrificed. The remaining 5 animals received an increase in dosage up to 40 mg. per kg. for another month before they were sacrificed. Gross and microscopic examinations were made as in the animals of *Group I*. Liver function, hematopoietic function, and renal function were evaluated before amithiozone was started, two weeks after commencing drug administration, and at monthly intervals thereafter.

The liver function studies consisted of bromsulfalein retention[4] studies (11), serum bilirubin determinations (12), and icteric indices (13). A control liver biopsy was obtained prior to drug administration.

The hematopoietic function studies consisted of total leukocyte counts, differential cell counts, hematocrit determinations, and erythrocyte sedimentation rates by the Wintrobe method.

The renal function studies (14) consisted of a determination of glomerular filtration rate (creatinine clearance), renal plasma flow (*p*-aminohippurate extractions using the Fich principle, $RPF = \frac{UV}{A-V}$), maximum tubular reabsorption of glucose (TmG), and microscopic examination of the urine. The *p*-aminohippurate extractions were determined, using a renal vein catheter which was inserted via the jugular vein under fluoroscopic guidance. The arterial blood was obtained from the femoral artery. The extraction of *p*-aminohippurate $\left(\frac{A-V}{V}\right)$ offered additional information for the evaluation of renal tubular function.

RESULTS

Acute toxicity

Group IA: Of the 7 dogs which received 100 mg. of amithiozone per kg. daily, 5 (71 per cent) died within forty-eight hours. Pleural effusion, edema of the mediastinum, and foamy white or pink exudate in the lumen of the tracheobronchial tree were constant findings in these animals. Pulmonary edema and passive congestion of the liver were consistent microscopic findings. In addition, one dog showed early bronchopneumonia. One dog which died within twenty-four hours showed small granulomatous areas in the pancreas unrelated to drug toxicity. The 2 dogs which did not die showed no significant findings.

Group IB: Of the 5 dogs in *Group IB* which received 50 mg. of amithiozone per kg., all survived the five-day period. At autopsy, one of the group had gross and microscopic edema of the lungs and mediastinum, bilateral pleural effusion, and intratracheal foamy exudate; 2 showed passive congestion of the liver. The remaining dogs in this group had no findings at autopsy attributable to amithiozone.

Chronic Toxicity

Group II: Of the 10 dogs started on 10 mg. of amithiozone per kg., one died within twenty-four hours and another after seven weeks of therapy. The remaining 8 dogs showed general good health until sacrificed. The dog which died acutely showed gross evidence of pulmonary edema and pleural effusion similar to the animals in *Group I* which received massive doses of the drug. The animal which

[4] Five mg. per kg. of body weight of bromsulfalein were given intravenously for this study.

died seven weeks after the start of drug administration was found to have heart worms (*Dirofilaria immitis*), bilateral pleural effusion, pulmonary edema, chronic passive congestion of the liver, and atrophy of the spleen. There was no evidence of primary damage to the liver, kidneys, or bone marrow. It was impossible to draw conclusions as to the cause of death in this animal. The autopsy findings strongly suggested that the parasites may have been an important factor. None of the animals in *Group II* which survived the five- and six-month study periods showed gross or histologic evidence of drug toxicity on the heart, lungs, pancreas, brain, or spleen.

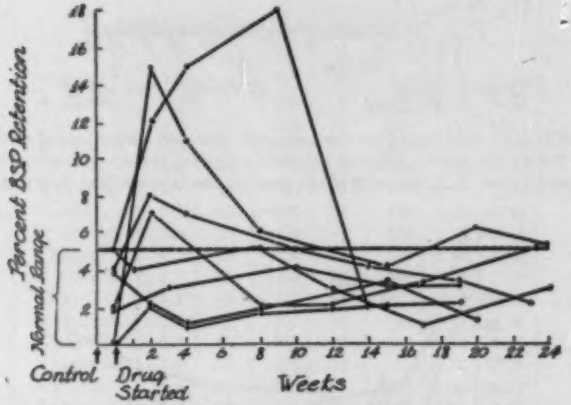

FIG. 1. Graph showing percentage of bromsulfalein retention during amithiozone administration to 8 normal dogs. This graph shows the impairment of liver function as reflected in the percentage of BSP retention over a six-month period. Four animals showed abnormal retention early in the course of drug administration. Nevertheless, this abnormality reverted to within normal limits in all cases despite continued drug administration.

Hepatic studies: Four animals developed bromsulfalein (BSP) retention above 7, but less than 18, per cent within two weeks after medication was started (figure 1). Examination of material obtained in control-study liver biopsies on 2 of these animals showed fatty infiltration. The abnormal retention of BSP returned to less than 5 per cent in all animals within fourteen weeks despite continued drug administration and an increase in dosage after three months. Bromsulfalein remained normal in the other dogs of *Group II*. Serum bilirubin (normal below 0.8 mg. per 100 cc.) and icteric indices showed no abnormalities in any of the dogs during this study. At the autopsy of the 8 dogs which survived the study period, no abnormalities relevant to amithiozone toxicity were noted. Even the 2 dogs which had previously shown fatty infiltration in material obtained by biopsy now showed complete regression on histologic examination.

Hematologic studies: The erythrocyte sedimentation rate decreased in all

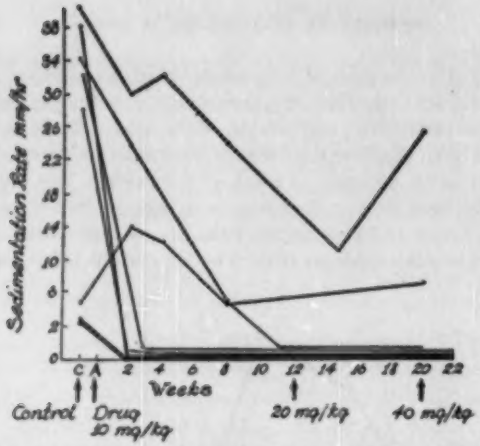

FIG. 2A. Effect of amithiozone on the sedimentation rate over a period of five months (*Group II*). The erythrocyte sedimentation rate decreased acutely following the administration of amithiozone. In 75 per cent of the animals the rate was less than one millimeter per hour.

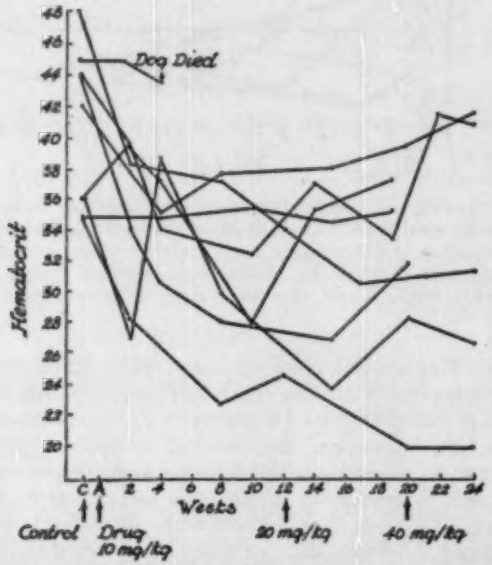

FIG. 2B. Effect of amithiozone on hematocrit (*Group II*). A tendency for the hematocrit to decrease in all animals which received amithiozone is shown. In the majority of cases the hematocrit stabilized at subnormal values. In approximately one-fourth to one-third of the animals, however, the hematocrit decreased progressively and constantly throughout the entire six-month period of amithiozone administration.

animals (figure 2A). This was marked during the first month in all but one dog. It is noteworthy that the sedimentation rate in 6 dogs decreased to less than one millimeter per hour and remained so until completion of the study.

A consistent decrease in the hematocrit (figure 2B) was noted in 7 of the 8 animals. The average decrease was 12 per cent. The maximal fall was noted between two and seventeen weeks after medication was started even though larger doses of amithiozone were administered after this period. Despite the

TABLE 1

Effect of Amithiozone on Renal Function*

DOG NUMBER	TIME	URINE	GFR†	RPF‡	PAH§ Ext.	TmG¶	GFR/TmG	FF¶	MICROSCOPIC URINALYSIS
		cc/min	*cc/min*	*cc/min*		*mg/min*			
1	Control	2.6	47	255	68	142	0.33	0.18	negative
	6 months	4.8	61	306	75	137	0.45	0.20	negative
5	Control	4.5	92	516	69	384	0.24	0.18	negative
	5 months	3.0	90	540	78	300	0.30	0.17	negative
8	Control	5.6	43	179	64	164	0.26	0.24	negative
	6 months	2.8	58	280	67	175	0.33	0.21	negative
10	Control	0.6	60	290	60	195	0.31	0.21	negative
	5 months	1.0	76	410	79	210	0.36	0.19	negative
12	Control	4.2	32	160	73	140	0.23	0.20	negative
	6 months	2.7	33	130	77	168	0.20	0.25	negative
15	Control	1.7	43	272	69	167	0.26	0.16	negative
	5 months	2.5	47	290	74	140	0.34	0.16	negative
16	Control	3.3	50	238	67	182	0.27	0.21	negative
	6 months	4.6	60	286	64	195	0.31	0.21	negative
20	Control	7.0	57	342	67	227	0.25	0.17	negative
	6 months	5.0	69	278	63	222	0.31	0.25	negative

* Dose: 10 mg. per kg. for three months and 20 mg. per kg. for the third to fifth months. Dogs numbers 1, 8, 12, 16, and 20 received 40 mg. per kg. for an additional month.

† GFR = glomerular filtration rate = creatinine clearance.

‡ RPF = renal plasma flow = $\frac{UV(PAH)}{A - V(PAH)}$.

§ *p*-aminohippurate (PAH) extraction = $\frac{A - V}{A}$

‖ TmG = tubular maximum for glucose = GFR × arterial concentration − UV.

¶ FF = filtration fraction = $\frac{GFR}{RPF}$.

decrease in hematocrit, the bone marrow was abnormal (erytheroid hyperplasia) in only one dog. There was no evidence of aplastic bone marrow or any overt findings which would reflect intravascular hemolysis.

Depression of the total leukocyte count was noted in 2 dogs in *Group II*. In one of these the count dropped from 5,100 to 3,800 per cu. mm. during the first two weeks of therapy. In the second dog the count dropped from 7,000 to 2,950 per cu. mm. during the seventeenth week of therapy even though the bone marrow showed myeloid hyperplasia. These values returned to normal despite continued drug administration. Seven dogs developed an eosinophilia between 10

and 32 per cent. In 4 of these, *Dirofilaria immitis* parasites were found at autopsy, which may have explained at least part of the eosinophilia.

Renal function studies: The renal function studies are summarized in table 1. Only the control values and the repeat studies done just before sacrificing the animals are included since the other monthly studies showed no pertinent differences. One may note that the glomerular filtration rate increased significantly in 5 of the 8 dogs. This was accompanied by an increase in renal plasma flow in 4. There was some evidence of reversible glomerular damage as reflected in intermittent albuminuria. However, this was not persistent in any instance. There was no evidence of renal tubular damage as reflected in the tubular transport of glucose (TmG) or in the efficiency of *p*-aminohippurate (PAH) extraction. Of the animals in *Group II* which survived the study period, histologic examination of the kidneys showed evidence of associated diseases but no evidence compatible with toxicity due to amithiozone. All of the observations seemed to indicate that increasing the dosage of amithiozone from 10 to 40 mg. per kg. had no additional toxic effect.

DISCUSSION

The high toxicity and mortality rate encountered in the dogs in *Group I* indicates that this drug is in itself quite toxic if given in large initial doses. Fatal toxicity over a wide dosage range is noteworthy. One dog died after receiving only 10 mg. of amithiozone per kg., whereas 2 of 7 dogs tolerated 100 mg. per kg. per day for five days without toxic manifestations. The findings at autopsy of pleural effusion and mediastinal and pulmonary edema (15), along with severe passive congestion of various organs in the absence of changes in the nervous system, suggest a direct effect of the drug on the vascular system, particularly in the lungs.

The fact that 4 dogs showed bromsulfalein retention which disappeared during therapy indicates that amithiozone may impair liver function in some cases. The impairment is neither severe nor permanent, however, since the values returned to normal despite continued drug administration which employed an increasing dosage schedule far in excess of amounts generally employed clinically. This would agree with Kalkoff as cited by Mertens and Bunge (2), who found that fatty degeneration of the liver appeared approximately ten weeks after patients were started on amithiozone but disappeared despite the continued administration of the drug. The fact that fatty degeneration of the liver, due to unknown causes, in 2 dogs cleared while these animals were on amithiozone further suggests that the drug does not have an outstanding hepatotoxic effect.

The erythrocyte sedimentation rate fell even in those dogs with normal premedication values. Other observers (16) have reported that amithiozone also has a specific effect on the erythrocyte sedimentation rate in man, irrespective of the type or severity of the disease process being treated. For example, patients with diseases such as carcinoma or leukemia which are known to be unresponsive to amithiozone showed a drop in erythrocyte sedimentation rate while taking the drug. Because of this, the sedimentation rate is useless for evaluation of the activity of tuberculosis during amithiozone therapy.

Amithiozone has been reported to produce both hemolytic (17) and aplastic anemia (18) in man. The decrease in the hematocrit in 7 of 8 dogs in the present study probably indicates the most serious toxic manifestation. It is difficult to explain in the absence of bone marrow changes or overt hemolysis. Perhaps the insensitivity of the tests employed or the chronicity of the study with slow progressive changes either of hematopoiesis or hemolysis militates against an evaluation of the type of process under way. The writers have noted a similar abnormality in 2 patients being treated for tuberculosis. In one of these the hematocrit dropped from 35 to 20 per cent over a period of two weeks without overt evidence of blood destruction. Sternal bone marrow appeared normal. The leukopenia which occurred in 2 of 8 dogs in *Group II* can be explained only by toxicity to amithiozone. This is in keeping with the observation that amithiozone causes agranulocytosis in man (2, 18).

Signs of renal damage have been reported in both children (19) and adults (20) who have received amithiozone. Because of this, an evaluation of renal toxicity seemed to be indicated. In the present study on dogs there appeared to be an increase in renal glomerular filtration rate above control levels. Increases in renal plasma flow paralleled the increases in glomerular filtration rate which in turn paralleled the appearance of anemia. Renal function studies and postmortem examinations suggested that the changes in renal function noted during amithiozone administration were most likely secondary to alterations in other organ systems of the body and were not due to primary renal damage.

The difference in toxicity between the acute studies and the chronic studies bears comment. In the chronic studies, the animals were started on smaller (10 mg. per kg.) doses of amithiozone which were gradually increased. In these circumstances the severe toxicity was reduced despite the fact that the dosage eventually approached that used in the acute studies. Even in the dogs showing evidence of hepatic damage, the process was reversible during continued medication. These observations suggest that tolerance to the drug develops (21) and that the best therapeutic regimen would be to start with small doses and then increase the dose until the desired daily intake has been obtained. The present study leads to the conclusion that the toxicity of amithiozone prohibits its use as a routine therapeutic measure in the treatment of tuberculosis. When other measures fail, however, a therapeutic trial of amithiozone may be worth the calculated risk. In such circumstances, patients who do not develop acute toxic manifestations from smaller doses of the drug (2 mg. per kg.) may withstand a graded dosage schedule on which quite a high intake may be attained without greatly increasing the incidence of prohibitive toxic reactions. The possibility of the development of tolerance by the infecting tubercle bacilli has not been considered in the present study.

SUMMARY

Amithiozone is a toxic drug, particularly if the initial dose is excessive. In dogs, this toxicity is less marked if small initial doses are used and the dosage subsequently gradually increased. In this way, a higher daily intake can be obtained with a minimum of toxic reactions. Because of toxicity, amithiozone is prob-

ably indicated only in those patients unresponsive to less toxic chemotherapeutic agents.

Amithiozone causes impairment of liver function as reflected in abnormal bromsulfalein retention. The abnormal values may return to normal, however, even though medication is continued.

Anemia is a rather consistent finding in dogs which receive amithiozone over a prolonged period. Severe anemia appears to be the most frequent and most serious toxic manifestation resulting from the long-term administration of this drug.

The erythrocyte sedimentation rate of dogs decreases progressively during amithiozone therapy. As a consequence, it would seem that the erythrocyte sedimentation rate would be useless as a tool in the evaluation of the activity of any disease process being treated.

Amithiozone exerts no adverse effect on the renal functions in dogs. It causes an increase in glomerular filtration rate and renal plasma flow. This is probably secondary to the hematologic changes.

SUMARIO

Estudios Experimentales sobre la Toxicidad de la Amitiozona

La amitiozona es una droga tóxica, en particular si la dosis inicial resulta excesiva. En los perros, esa toxicidad es menos pronunciada si se emplean pequeñas dosis iniciales y se va aumentando paulatinamente la dosificación. De ese modo, cabe obtener una absorción diaria mayor con un mínimo de reacciones tóxicas. Debido a su toxicidad, la amitiozona probablemente sólo está indicada en los enfermos que no responden a agentes quimioterapéuticos menos tóxicos.

La amitiozona afecta la función hepática, según refleja la retención anormal de BSF. Esta puede volver a lo normal, aun continuando la medicación.

La anemia es un hallazgo bastante constante en los perros que reciben amitiozona durante un período prolongado. La anemia intensa parece ser la más frecuente y grave manifestación tóxica consecutiva a la administración de la droga a largo plazo.

El índice de sedimentación baja progresivamente hasta en los animales sanos mientras reciben la droga. Esto parece ser anespecífico e inutiliza la eritrosedimentación como instrumento para justipreciar la actividad de cualquier proceso patológico en vías de tratamiento.

No hay efecto adverso sobre la función renal en los perros. Se observa sí aumento de la velocidad de la filtración glomerular y del paso del plasma renal. Esto es probablemente secundario a las alteraciones sanguíneas.

RESUME

Etude expérimentale sur la toxicité de l'amithiozone

L'amithiozone est un médicament toxique, en particulier lorsque la dose initiale est excessive. Chez le chien, cette toxicité est moins marquée quand la dose initale administrée est faible et que les doses suivantes sont augmentées

progressivement. Ceci permet l'administration d'une dose quotidienne plus élevée qui peut être absorbée avec un minimum de réaction toxique. En raison de sa toxicité il est sans doute préférable de réserver l'administration de l'amithiozone aux malades qui ne répondent pas aux agents chimiothérapeutiques moins toxiques.

L'amithiozone détermine un trouble de la fonction hépatique comme il est démontré par la rétention de bromosulfaléine, mais le retour à la normale peut se produire même lorspue la médication est continuée.

L'anémie est rencontrée de façon presque habituelle chez les chiens recevant l'amithiozone pendant longtemps. L'anémie grave apparaît comme la manifestation de toxicité la plus fréquente et la plus sérieuse qui résulte de l'administration à long terme de ce médicament.

La vitesse de sédimentation chez les chiens décroît progressivement quand cette drogue leur est administrée. Ce phénomène n'est pas spécifique et rend le taux de sédimentation inutile comme moyen d'évaluation de l'activité de tout processus pathologique traité.

Chez le chien on n'observe pas d'effet nocif sur la fonction du rein. Le produit détermine une augmentation du taux de filtration glomérulaire et du débit plasmatique dans le rein. Ceci est probablement secondaire à des modifications qui se produisent dans le sang.

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A TUBERCULOSTATIC SERUM SUBSTANCE POSSESSING LYSOZYME-LIKE PROPERTIES[1,2]

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INTRODUCTION

In the course of work on evaluation of immune factors operating in tuberculosis, it was observed that the sera of rabbits immunized with heat-killed tubercle bacilli in Freund's adjuvants (mineral oil and Falba) are bacteriostatic for the H37Rv strain of *Mycobacterium tuberculosis* var. *hominis*. The sera of some tuberculous humans are also tuberculostatic (1).[4] There are very few reports in the literature on the tuberculostatic activity of the sera of tuberculous humans or animals. Pagel (2, 3) noted that tuberculostasis frequently occurred in glycerol broth and in Kirchner's medium when the serum of tuberculous patients was added, especially patients with chronic miliary tuberculosis, a type generally regarded as being associated with high resistance. The active principle was observed to be heat stable and present in approximately 10 per cent of normal human sera. It was not found in animal sera even when the animals were made tuberculous by infection or vaccination. The inactivity reported for animal sera may have been due to the relative insensitivity of the test as conducted with *M. tuberculosis* as well as to low concentrations of active substance which may have been present. Pagel considered the substance to be nonspecific, principally because he succeeded in absorbing it from serum with suspensions of *Escherichia coli*. Lurie's (4, 5) observation that the body fluids in immunized rabbits and guinea pigs are bacteriostatic for tubercle bacilli indicates a similar substance.

Additional communications on nonspecific antibacterial serum substances include the reports of Mackie and Finkelstein (6) and Mackie, Finkelstein, and Van Rooyen (7) on a nonspecific bactericidin of rabbit serum, which is especially active against gram-positive bacteria. Wulff (8) described a nonspecific thermostable substance in the serum of febrile patients which is bactericidal for some strains of *Neisseria meningitidis*. Jacox (9) also reported on a nonspecific substance bactericidal for *Bacillus subtilis* which appears in the serum of humans in the acute phase of a variety of diseases. The material was reported to require calcium for its activation and was most active at pH values near neutrality. Dubos (10) has extracted a substance from various animal tissues which exerts

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[2] This work has been supported (in part) by the State of Washington Funds for Medical and Biological Research, and (in part) by the Alice McDermott Research Foundation of the University of Washington.

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[4] The term "tuberculostatic" is used to indicate bacteriostasis for the tubercle bacillus.

a pronounced bacteriostatic effect on tubercle bacilli. However, he does not believe that it is lysozyme.

MATERIAL AND METHODS

The present investigations on the characterization of the tuberculostatic substance in serum were made with the sera of rabbits which had been given a series of three weekly subcutaneous doses of 60 mg. of heat-killed tubercle bacilli in Freund's adjuvants (11). As shown later, the serum of normal rabbits contains very little of the active substance.

Bacterial inhibition tests were conducted with young cultures of nonpathogenic microorganisms by heavily inoculating nutrient broth and nutrient agar (0.5 per cent agar), containing varying amounts of crystalline egg-white lysozyme[4], and the serum in twofold dilutions ranging from 1:2 to 1:512, and incubating at 37°C. for twelve to twenty-four hours. A susceptible parent strain of *Sarcina lutea* was used for the routine determination of the lysozyme activity of tuberculostatic sera and of egg-white lysozyme. The resistant strain of *S. lutea* was developed by growing the parent strain in nutrient agar containing increasing quantities of egg-white lysozyme. Glycerol agar containing 2 per cent glycerol and 0.5 per cent agar was used for bacteriostatic tests with the *Mycobacteria*, and incubation was carried out at 37°C. for one to two weeks. The tests were conducted in small capped vials, and the minimum concentration of test substance showing complete inhibition of growth was taken to represent the inhibiting titer. The inhibiting titers were expressed in γ per ml. in the case of egg-white lysozyme and with the substance in serum as lysozyme equivalents. A lysozyme equivalent was selected to be that amount of the substance in serum possessing bacteriostatic activity against *S. lutea* equivalent to that exerted by 1 γ of egg-white lysozyme.

The carbohydrate used for the precipitin test was prepared by extracting tubercle bacilli for six hours at 100°C. with 8 per cent sodium hydroxide in 4 per cent sodium acetate. The extract was neutralized with hydrochloric acid, filtered, precipitated with 5 volumes of alcohol, dissolved in water, reprecipitated with alcohol, dried, and finally dissolved in saline.

EXPERIMENTAL RESULTS

The substance in serum was found to possess properties which are very similar to those of egg-white lysozyme. When the serum was treated by the alcohol fractionation method of Cohn (12) at 0°C., the active principle came down largely in the 30 to 45 per cent alcohol fraction and was readily soluble in water. For example, when serum was fractionated and each respective fraction dissolved in a volume of water one-fifth that of the original serum, the activities in lysozyme equivalents per ml. were as follows: 0 to 30 per cent fraction, none; 30 to 45 per cent fraction, 300; 45 to 50 per cent fraction, none. In common with egg-white lysozyme, the heat stability of the substance in serum is greatest at low pH values. When the pH of the serum was adjusted to 6.0 with 0.1 normal hydrochloric acid and compared with unadjusted serum at pH 8.0, it was observed that the activity of the former was unaffected by heating at 65°C. for thirty minutes; whereas the latter was completely inactivated in fifteen minutes at the same temperature. All activity against *S. lutea* and the BCG

[4] The crystalline egg-white lysozyme used throughout this work was generously supplied by Armour and Co., Chicago, Ill.

strain of *M. tuberculosis* was removed when the serum was diluted 1:2 with physiologic saline and treated for five minutes by the addition of 1 per cent bentonite. This observation indicates that the substance in serum has adsorption affinities similar to lysozyme.

Apparently, the activity of the serum is not due to specific antibody, since only partial loss of activity resulted when the serum was diluted with an equal volume of saline and absorbed with heat-killed *M. tuberculous* H37Rv by treatment at 4°C. with four successive one-tenth volume doses of saline-washed organisms over a period of twenty-four to forty-eight hours. For example, a

TABLE 1

Antibacterial Spectrums of Egg-White Lysozyme and Tuberculostatic Serum

TEST ORGANISM	INHIBITING TITER EGG-WHITE LYSOZYME IN γ PER ML.	INHIBITING TITER	
		Serum lysozyme equivalents per ml.*	Actual serum dilution
E. coli	resistant†	resistant†	resistant†
Corynebacterium diphtheriae	resistant†	resistant†	resistant†
M. tuberculosis avium	resistant†	resistant†	resistant†
Mycobacterium smegmatis	resistant†	resistant†	resistant†
Pseudomonas aeruginosa	resistant†	resistant†	resistant†
Micrococcus lysodeikticus	0.3	0.3	1:400
BCG strain of *M. tuberculosis* var. *bovis*	5.0	7.5	1:16
M. tuberculosis H37Ra	10.0	8.5	1:14
M. tuberculosis H37Rv	12.0	12.0	1:10
Mycobacterium phlei	5.0	7.5	1:16
S. lutea (parent)	0.3	0.3	1:400
S. lutea (egg-white lysozyme resistant)	1.5	1.2	1:100

* A lysozyme equivalent represents activity equal to 1 γ of egg-white lysozyme as defined under "Materials and Methods."

† Resistant = no inhibition by 100 to 200 γ per ml. of egg-white lysozyme or 120 serum lysozyme equivalents per ml.

serum possessing original activity of 100 lysozyme equivalents per ml. was reduced to only 75 lysozyme equivalents per ml. by such treatment. At the same time the procedure completely removed the precipitins against carbohydrates of the tubercle bacillus which were originally present in a titer of 1:500,000. As further evidence that the active principle is not an antibody, it was observed that the heat-inactivated sera of 2 rabbits immunized with *Escherichia coli* in Freund's adjuvants showed an activity of 50 lysozyme equivalents per ml. against *S. lutea* and no activity against *E. coli*. Increases in serum activity against *S. lutea* and *M. tuberculosis* were also noted when rabbits were immunized with other organisms such as *M. smegmatis* and *Saccharomyces cerevisiae* in Freund's adjuvants. When egg-white lysozyme and the serum were dialyzed in commercial cellophane membranes for twenty-four hours against saline at 4°C., approximately 30 per cent of the activity of both substances was lost to the dialysate. The activity of serum was also suppressed by menstruums of high

ionic strength in a manner similar to that observed for lysozyme (13). For example, the minimum concentration of calcium chloride necessary to accomplish complete suppression of the activity of either 10 γ per ml. of egg-white lysozyme or 10 lysozyme equivalents per ml. of serum was found to be 0.05 M. Results have also been obtained which indicate that calcium is not necessary for the activation of either substance (1). Still further evidence of the lysozyme-like character of the substance in serum is the similarity of the antibacterial spectrums of the two materials as shown in table 1. The results presented were obtained with an immune rabbit serum containing 120 lysozyme equivalents per ml. The sera of several other rabbits gave similar spectrums.

DISCUSSION

The results show that there is a good correlation between growth inhibition of the various organisms by egg-white lysozyme and the tuberculostatic substance in serum. The observation that the egg-white lysozyme-resistant strain of *S. lutea* shows approximately equal resistance to the bacteriostatic action of serum, and egg-white lysozyme provides strong evidence that the active substance in the serum is lysozyme-like. This is in accord with the observation of Fleming (14) who found that, when organisms are cultivated to develop resistance to the lytic action of lysozyme of one species of animal, they acquire equal resistance to the lysozyme of other species. In the present work, characteristic lysis of *Micrococcus lysodeikticus* and *S. lutea* was observed at the minimum inhibiting concentrations of both the serum and egg-white lysozyme; whereas lysis was never noted with any of the gram-negative bacteria or the *Mycobacteria* tested. Wolff (15) reported that tubercle bacilli are killed, but presumably not lysed, by crude lysozyme concentrates of egg white.

The marked susceptibility of *S. lutea* and *Micrococcus lysodeikticus* and apparent insusceptibility of *M. smegmatis* and *M. tuberculosis avium* to the two substances is notable. It is also to be noted that tubercle bacilli of the BCG strain are more susceptible than those of the H37 strains. Tests on the sera of some 50 or more normal rabbits have shown very little activity. For example, tests with the sera of one group of 8 normal rabbits showed that all contained less than 2.0 lysozyme equivalents per ml. and that 2 were entirely without activity. In contrast to this, the sera of rabbits immunized with heat-killed tubercle bacilli in Freund's adjuvants frequently showed as many as 120 lysozyme equivalents per ml. The calculated mean value of the sera of 12 rabbits immunized with tubercle bacilli in Freund's adjuvants was 80 lysozyme equivalents per ml.

Reports of the presence of lysozyme in animal serum were first made by Fleming (16), Fleming and Allison (17), and, more recently, by Smolens and Charney (18). However, little attention has been given to the lysozyme levels in serum in relation to stress and disease.

The present observation that rabbits heavily immunized with heat-killed tubercle bacilli in Freund's adjuvants possess a serum titer of lysozyme equivalents approximately ten times greater than the minimum amount necessary to produce *in vitro* bacteriostasis of *M. tuberculosis* H37Rv raises the question

whether or not this lysozyme-like substance may play a role in resistance to tuberculosis.

SUMMARY

Strong tuberculostatic activity is present in the serum of rabbits immunized with heat-killed tubercle bacilli in Freund's adjuvants. The active principle in rabbit serum appears to act independently of antibodies and complement. It is similar to egg-white lysozyme in its heat stability, adsorption behavior, inhibition by salts, dialyzability, and antibacterial spectrum. The BCG strain of *M. tuberculosis* is more susceptible to egg-white lysozyme and the tuberculostatic serum substance than the H37 strains of *M. tuberculosis*. In contrast, *M. tuberculosis avium* and *M. smegmatis* are apparently completely resistant to these substances.

SUMARIO

Substancia Sérica Tuberculostática Dotada de Propiedades Lisozimoideas

En el suero de los conejos inmunizados con bacilos tuberculosos matados al calor en los coadyuvantes de Freund, existe potente actividad tuberculostática. El principio activo en el suero de conejo parece obrar independientemente de los anticuerpos y del complemento. Es semejante al lisozima de la clara de huevo en su termoestabilidad, comportamiento sorbente, inhibición por sales, dializabilidad y espectro antibacteriano. La cepa BCG del *M. tuberculosis* es más susceptible al lisozima de la clara de huevo y a la substancia sérica tuberculostática que las cepas H37 del *M. tuberculosis*. En contraposición, el *M. tuberculosis avium* y el *M. smegmatis* son al parecer absolutamente resistentes a dichas substancias.

RESUME

Sérum contenant une substance tuberculostatique possèdant des propriétés analogues à celles de la lysozyme

Une forte activité tuberculostatique est présente dans le sérum de lapins immunisés par des bacilles tuberculeux tués dans un milieu contenant les adjuvants de Freund. Le principe actif du sérum de lapin semble agir indépendamment des anticorps et du complément. Ses propriétés sont analogues à celles de la lysozyme du blanc d'oeuf quant à la stabilité à la chaleur, les caractéristiques de l'absorption, l'inhibition par les sels, la dialyzabilité et l'étendue de son action antibactérienne. La souche BCG du *M. tuberculosis* est plus sensible à la lysozyme du blanc d'oeuf et à la substance bactériostatique du sérum que les souches H37 du *M. tuberculosis*. Par contre, le *M. tuberculosis avium* et le *M. smegmatis* semblent entièrement résistants à ces substances.

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A NEW SEROLOGIC TEST FOR TUBERCULOSIS[1]

Preliminary Report

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INTRODUCTION

A serologic test which gives accurate quantitative information would be a valuable aid in the diagnosis and management of tuberculosis. Middlebrook and Dubos (1) described a specific hemagglutination reaction between sera of tuberculous patients and sheep cells treated with a polysaccharide fraction of mammalian tubercle bacilli. Other investigators (2, 3) confirmed this observation, using a preparation of Old Tuberculin as the antigen. Middlebrook (4) more recently added complement to the hemagglutination test and read the results in terms of hemolysis.

The purpose of this paper is to report on a serologic test which gives quantitative information. Quantitative results, to be of value, must be reproducible. This requires that the reagents and technique be quantitatively standardized since the dilution of the patient's serum alone may not give accurate quantitative results and consequently may not be reproducible.

MATERIALS AND METHODS

Patient's serum: Approximately 10 ml. of blood were collected from each patient and, after clotting, the serum was removed. The serum was inactivated in a water bath at 56°C. for thirty minutes. The inactivated serum was absorbed with sheep erythrocytes to remove antibodies unrelated to tuberculosis which may produce hemolysis. The absorption was accomplished by adding 0.4 ml. of packed sheep erythrocytes to each 1 ml. of the patient's serum, allowing the mixture to stand for twenty minutes at 3° to 6°C. An alternate method of absorption was to add 0.2 ml. of packed sheep cells to each 1 ml. of the patient's serum, allowing the mixture to stand overnight at 3° to 6°C. It was then centrifuged for five minutes at 2,000 r.p.m., and the clear serum was separated from the cells. The largest amount used in the test was 0.1 ml. of patient's serum absorbed with sheep cells, since it was determined that larger amounts of low reacting tuberculous sera did not show any appreciable increase in the reactivity.

Washed packed sheep erythrocytes: Sheep's blood collected in Alsever's (5) solution was filtered through a double layer of cheese cloth into a centrifuged tube and at least five times the quantity of physiologic saline (0.85 per cent) was added. The cells were washed three times. At the last washing, the cell suspension was centrifuged at 2,000 r.p.m. for twenty minutes to adequately pack the cells. The supernatant fluid was removed. These packed sheep cells were used for the preparation of the antigen and for the absorption of the patient's serum.

Antigen: Sheep cells were treated with Old Tuberculin[2] in the following manner. Three different concentrations of OT, 1:8, 1:10, and 1:12, were obtained by diluting with Eagle's

[1] From the Division of Laboratories and Research, Nassau County Department of Health, Hempstead, New York.

[2] Concentrated tuberculin (4 times international standard, lot No. 2725-19A) was kindly supplied by Dr. Piersma of Lederle Laboratories, Pearl River, New York.

(6) buffered saline pH 7.2. To various volumes of each of these concentrations of tuberculin, 0.1 ml. of packed sheep cells was added. The mixtures of tuberculin and sheep cells were incubated in the water bath at 37°C. for two hours. The tubes were shaken at intervals of

TABLE 1

Standardization of Antigen

Determination of Optimum Concentration of Tuberculin and the Relation of the Tuberculin Concentration to the Sheep Cells

CONCENTRATION OF OLD TUBERCULIN	PROPORTION OF TUBERCULIN CONCENTRATION TO SHEEP CELLS	LOW REACTING SERUM DILUTION OF SERUM			MODERATE REACTING SERUM DILUTION OF SERUM						HIGH REACTING SERUM DILUTION OF SERUM							
		Undiluted	1:2	1:3.3	1:3.3	1:5	1:6.6	1:10	1:12.5	1:16.6	1:25	1:33.3	1:50	1:66	1:100	1:125	1:166	1:250
		Degree of Hemolysis			Degree of Hemolysis						Degree of Hemolysis							
1:8	1:40	80	25	15	95	75	30	15	0	0	100	100	100	100	90	75	45	
	1:60	100	40	15	100	100	95	85	40	20	100	100	100	100	100	100	85	30
	1:80	100	45	20	100	100	95	85	35	20	100	100	100	100	100	100	85	40
	1:100	65	20	0	95	90	75	20	5	0	100	100	100	100	100	75		
1:10	1:40	65	20	0	100	100	80	50	25	10	100	100	100	100	95	85	50	25
	1:60	75	25	0	95	75	45	15	0	0	100	100	100	100	85	70	20	0
	1:80	80	15	0	100	90	75	30	5	0	100	100	100	100	95	85	25	10
	1:100	50	15	0	90	80	55	15	0	0	100	100	100	95	85	70	25	15
1:12	1:40	35	0	0	100	100	80	35	20	0	100	100	100	100	95	80	25	
	1:60	50	10	0	100	100	95	40	20	5	100	100	100	100	95	85	40	15
	1:80	30	0	0	100	95	80	25	15	0	100	100	100	95	85	40	25	
	1:100	30	0	0	100	85	45	25	0	0	100	100	95	85	40	25	5	

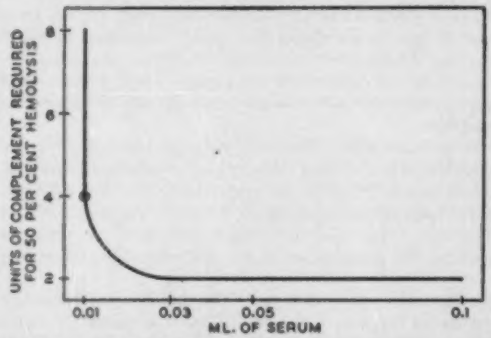

FIG. 1. Determination of optimum amount of complement for the test

fifteen minutes. The treated cells were washed four times with physiologic saline, and saline was added to the washed packed cells to make a cell suspension of 2.5 per cent. The treated cells were then tested in the presence of low, moderate, and high reacting tuberculous sera

and four units of complement. The results of the test indicated that optimum conditions were obtained with sheep cells which were treated in a proportion of one part of cells to 80 parts of 1:8 concentration of OT. A 2.5 per cent suspension of these cells in saline was used as antigen (table 1).

Complement: Dried, frozen complement was used in this test. The complement was reconstituted, a dilution of 1:40 was made, and the amount required for 50 per cent hemolysis (7) was determined. This amount was considered to be a unit of complement. The antigen preparation which was found optimum was tested with two, four, six, and eight units of complement in the presence of a moderately reacting serum. The point of 50 per cent hemolysis for each amount of complement was plotted against the amount of tuberculous serum, and a curve similar to that noted in an amboceptor titration was obtained. Four units of complement were determined to be the optimum amount since the maximum reaction was obtained with the smallest amount of tuberculous serum. Larger amounts of complement did not appreciably increase the degree of reactivity (figure 1).

TABLE 2

Technique of Preliminary Test

REAGENT	AMOUNT
	ml.
Patient's serum	0.1
Physiologic saline	0.1
Antigen (tuberculin-treated sheep cells)	0.2
Complement—four units	0.1
Total	0.5

OBSERVATIONS

Technique of the Preliminary Test

Specimens were examined by a preliminary test which consisted of 0.1 ml. of the patient's serum, four units of complement contained in 0.1 ml., 0.2 ml. of 2.5 per cent suspension of antigen (tuberculin-treated sheep cells), and 0.1 ml. of physiologic saline. The total volume was always 0.5 ml. (table 2).

The following controls were included:

A known reacting tuberculous serum and a nonreacting serum; a control for anticomplementary property of the patient's serum, consisting of 0.1 ml. of the patient's serum, one unit of complement contained in 0.1 ml., 0.1 ml. of appropriate dilution of anti-sheep amboceptor (8), 0.1 ml. of 2.5 per cent suspension of sheep cells, and 0.1 ml. of physiologic saline.

The tests were allowed to stand for one hour at 3° to 6°C. They were shaken to resuspend the cells, placed in a water bath at 37°C. for thirty minutes, and shaken at one-minute intervals. The results were read by comparing the various degrees of hemolysis in the tests to a color standard representing degrees of hemolysis from 0 to 95 per cent at intervals of 5 per cent (9). Any serum with which no hemolysis was noted was considered to be nonreacting and no further tests were done.

Technique for the Quantitative Determination of Tuberculous Antibodies in Reacting Sera

Sera which gave evidence of a reaction in the preliminary test were further tested in order to determine the titer. The sera with which partial degrees of hemolysis were obtained were retested in four amounts 0.1, 0.05, 0.03, and 0.02 (tubes 1–4, table 3). The sera with which complete hemolysis occurred were retested in ten amounts from 0.1 to 0.003 and are represented in tubes 1–10 (table 3).

TABLE 3

Technique for Test with Reacting Serum

		TUBE									
		1	2	3	4	5	6	7	8	9	10
PATIENT'S SERUM	Dilution used	Undiluted	Undiluted	Undiluted	Undiluted	1:4	1:10	1:10	1:10	1:10	1:10
	Amount pipetted	ml. 0.1	ml. 0.05	ml. 0.03	ml. 0.02	ml. 0.05	ml. 0.1	ml. 0.08	ml. 0.06	ml. 0.04	ml. 0.03
	Final dilution represented	—	1:2	1:3.3	1:5	1:6.6	1:10	1:12.5	1:16.6	1:25	1:33.3
Physiologic saline		0.1	0.15	0.17	0.18	0.14	0.1	0.12	0.14	0.16	0.17
Antigen—tuberculin-treated sheep cells		0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Complement—4 units		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

The following controls were included:

A known reacting tuberculous serum and a nonreacting serum; a control for incomplete absorption of heterophil antibodies consisting of 0.1 ml. of the patient's serum, four units of complement contained in 0.1 ml., 0.2 ml. of 2.5 per cent suspension of untreated sheep cells, and 0.1 ml. of physiologic saline; a control for anticomplementary property of the patient's serum consisting of 0.1 ml. of the patient's serum, one unit of complement contained in 0.1 ml., 0.1 ml. of appropriate dilutions of anti-sheep amboceptor (8), 0.1 ml. of 2.5 per cent suspension of sheep cells, and 0.1 ml. of physiologic saline.

The tests were allowed to stand for one hour at 3° to 6°C. They were then shaken to resuspend the cells, placed in a water bath at 37°C. for thirty minutes, and shaken at one-minute intervals. The results were read by comparing the various degrees of hemolysis in the tests to a color standard representing degrees of hemolysis from 0 to 95 per cent at intervals of 5 per cent (9). If the end point was still not reached, higher dilutions of the patient's sera were tested. The control for incomplete absorption of heterophil antibodies with untreated sheep cells should show no hemolysis; the control for anticomplementary property of the serum should show partial hemolysis.

Calculation of Titer

The minimal amount of patient's serum giving 50 per cent hemolysis in the presence of tuberculin-treated sheep cells was considered to represent the end

result, and the titer of the serum was the dilution in which this reaction occurred. If 50 per cent hemolysis was not obtained with any of the serum dilutions in the test, the titer was approximated to the nearest point of 50 per cent hemolysis.

Example: If 50 per cent hemolysis was obtained with 1:10 dilution of the patient's serum, the titer was 10. If, however, 65 per cent hemolysis was obtained with 1:10 dilution and 40 per cent hemolysis with 1:12.5 dilution of the patient's serum, the titer was recorded as 11.

TABLE 4

Comparison of Titers of Sera from Tuberculous and Nontuberculous Donors

Donors	Approximated titers													
	No reaction	1	2	4	8	14	18	28	38	45	60	70	200	Totals
Tuberculous	4	6	9	13	4	6	4	4	3	1	6	2	1	63
Nontuberculous														
Pregnant women	125													125
Employees	33													33
Patients with following diagnoses:														
Syphilis	19	1												20
Cancer	12	2												14
Coronary disease	4	2	1											7
Bronchopneumonia	6													6
Coccidioidomycosis	1													1
Miscellaneous diseases	73													73

Sera Tested from Tuberculous and Nontuberculous Donors

Nontuberculous sera: Sera were tested from 158 apparently normal persons, all of whom were examined roentgenographically and were found to show no evidence of any infiltrative disease of the lungs. No reactions were obtained with any of these specimens. Sera from 121 patients with miscellaneous diseases were tested, and a titer of one was obtained with 5 and a titer of two with another. The remaining 115 specimens gave no reactions.

Tuberculous sera: Sera from 63 patients with clinical and laboratory evidence of tuberculosis were tested. No reactions were obtained with specimens from 4; the sera from the remaining 59 displayed the reaction in titers of 1 to 200 (table 4).

In order to determine whether these titers were reproducible, sera from 22 tuberculous patients were tested on two separate days. The titers obtained with the serum from the same patient tested at different times closely approximated each other. The titers ranged from 1 to 60.

SUMMARY

A serologic reaction of serum from tuberculous patients is described. The hemolytic reaction which characterizes the test results from the fixation of

complement by an antigen antibody system. The specific complex is presumably formed at the cell surface and, in the presence of complement, lysis of erythrocytes occurs similar to lysis by specific hemolysin. The binding of specific antibody as well as complement to the surface of the blood cells is presumably determined by preliminary coating of the cell surface with antigen. The titer of the tuberculous antiserum is determined as the highest dilution which causes a constant degree of hemolysis (50 per cent) with a constant dose of complement (4 units).

This procedure may be useful in the study of other infectious diseases provided suitable antigens are made available.

SUMARIO

Nueva Serorreacción para Tuberculosis: Informe Preliminar

La reacción hemolítica que caracteriza a esta prueba proviene de la fijación del complemento por un sistema de antígeno-anticuerpo. El complejo específico se forma en la superficie de los glóbulos y en presencia del complemento, ocurre lisis de los hematíes, semejante a la producida por hemolisina específica. La fijación del complemento a la superficie de los hematocitos se determina por el recubrimiento preliminar de la superficie de los glóbulos con antígeno. El título del antisuero tuberculoso se define como la dilución máxima que provoca una hemólisis de magnitud constante (50 por ciento) con una dosis constante de complemento.

Este procedimiento puede resultar útil en el estudio de otras enfermedades infecciosas, con tal que se cuente con antígenos adecuados.

RESUME

Un nouveau test sérologique pour la tuberculose: rapport préliminaire

La réaction hémolytique qui caractérise le test résulte de la fixation du complément par un système antigène-anticorps. Le complexe spécifique est formé à la surface de la cellule et, en présence du complément, il se produit une hémolyse semblable à celle qui est déterminée par une hémolysine spécifique. La fixation de l'anticorps spécifique comme celle du complément, à la surface des cellules du sang, est déterminée par l'enrobage préliminaire de la surface du globule par l'antigène. Le titre de l'antisérum tuberculeux est déterminé comme étant la dilution la plus élevée qui produit un degré constant d'hémolyse (50%) avec une dose constante de complément (4 unités).

Ce procédé pourrait se montrer utile pour l'étude d'autres maladies infectieuses si des antigènes convenables devenaient disponibles.

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Case Reports

REACTION TO PARA-AMINOSALICYLIC ACID SIMULATING TUBERCULOUS MENINGITIS[1]

EZRA V. BRIDGE AND GERALD N. HOFMANN

(Received for publication August 8, 1951)

When the oral administration of para-aminosalicylic acid (PAS) is followed by fever, headache, stiff neck, and increased cerebrospinal fluid cell count in a patient with tuberculosis, a report of the case seems justified. It is believed by the writers that the neurologic reaction represented a manifestation of PAS toxicity and is the first recorded instance in which such a toxic reaction simulated tuberculous meningitis. The importance of such a type of reaction is readily appreciated when it is considered that tuberculous meningitis requires early, vigorous treatment and that intravenous PAS is frequently a part of this treatment. Certainly, if the patient is already intensely sensitive to PAS following oral administration, intravenous medication might well be lethal. Moreover, any drug reaction which simulates so serious a complication as tuberculous meningitis makes diagnostic and therapeutic decisions concerning the primary disease considerably more difficult.

CASE REPORT

E. G. N., a 24-year-old white woman, entered Mineral Springs Sanatorium on January 27, 1951 with minimal pulmonary tuberculosis. Her sputum was positive for tubercle bacilli by gastric culture. One brother died of tuberculous meningitis three months before her admission to the Sanatorium.

Because of a mild spread in her pulmonary disease, oral PAS, 12 gm. daily, and intramuscular dihydrostreptomycin, 1 gm. twice weekly, were started on the one hundred and eighteenth hospital day. Within a day or two she began to complain of mild headache and generalized muscular aches and pains.

On the one hundred and twenty-sixth hospital day, after eight days of chemotherapy, she had a rigorous chill. Her temperature increased to 102°F. The next day she had more headache, backache, another chill, an increase in temperature to 103.8°F., and a stiff neck. No leg signs could be elicited.

Because of these symptoms, she did not take PAS during the next two days and her temperature did not exceed 99.4°F.

On the one hundred and thirtieth hospital day (twelve days after start of PAS therapy) she took 4 gm. of PAS and shortly thereafter had a severe chill, a rise in temperature to 105.8°F., severe headache, backache, stiff neck, a diffuse, nonpruritic, maculopapular rash, and leukopenia with lymphocytosis. The rash vanished as her fever declined.

The following morning the cerebrospinal fluid showed 49 cells per cu.mm., all lymphocytes, 20 mg. of protein per 100 cc., 66 mg. of sugar per 100 cc., and 712 mg. of chlorides per 100 cc. PAS was withheld for five days, but dihydrostreptomycin was increased to 1 gm. daily. Her temperature generally remained subnormal. Her total leukocyte count increased but lymphocytosis was still evident.

On the morning of the one hundred and thirty-sixth hospital day the cerebrospinal fluid showed 10 cells, all lymphocytes, 80 mg. of protein per 100 cc., 62.5 mg. of sugar per 100 cc.,

[1] From the Mineral Springs Sanatorium, Cannon Falls, Minnesota.

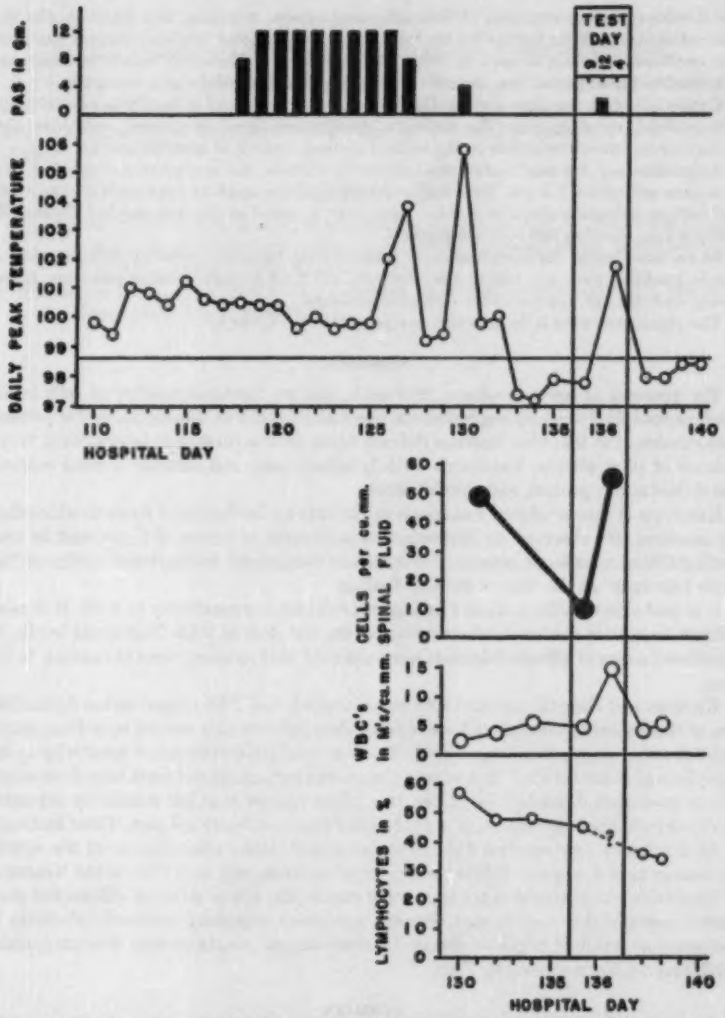

FIG. 1. This correlates the development of intermittent fever and changes in the cerebrospinal fluid cell count and total leukocyte count with PAS therapy. Attention is directed to the normal temperature and cerebrospinal fluid cell count before administration of 2 gm. of PAS on the test day, with increase in both after the drug was given.

and 740 mg. of chlorides per 100 cc. The total leukocyte count was 4,600 per cu.mm. with 45 per cent lymphocytes. At noon the patient was given a test dose of 2 gm. of PAS by mouth. Shortly thereafter she had a mild chill and her temperature increased to 101.8°F.

She developed severe cramping abdominal pains, nausea, vomiting, and diarrhea. She disclaimed headache. Four hours after the test dose of PAS, another lumbar puncture was done. The cerebrospinal fluid showed 54 cells, all lymphocytes. The total leukocyte count had increased to 14,950 per cu.mm. but, unfortunately, no differential count was made.

On the following two days the total leukocyte count was found to be within normal limits with a slight lymphocytosis. The patient's temperature remained normal, and there were no further symptoms referable to the central nervous system or gastrointestinal tract.

Approximately five weeks after the last febrile episode, she was given a single test dose of sodium salicylate, 1.3 gm., followed in a week by 2 gm. each of para-aminobenzoic acid and sodium salicylate given in divided doses over a period of two and one-half hours. She exhibited no reaction to these compounds.

At no time during the foregoing experiences did she have photophobia. Although manometric readings were not taken, the cerebrospinal fluid at each lumbar puncture flowed slowly and did not appear under increased pressure.

The significant events in this case are presented in figure 1.

COMMENT

The presence of fever, headache, stiff neck, and an increased number of cells in the cerebrospinal fluid strongly suggested the onset of tuberculous meningitis in this patient. Nevertheless, the following features did not favor such a diagnosis: intermittent fever, absence of photophobia, leukopenia with lymphocytosis, and initially normal cerebrospinal fluid sugar, protein, and chloride levels.

Randolph (1) has produced leukopenia in patients by feeding them foods to which they are sensitive. He observed no depression of leukocytes in excess of 5 per cent in test-feeding fifteen nonallergic persons. He considers "trajectory leukocytosis" a sign of "allergic tolerance" at the time of the test-feeding.

It is probable that the patient's leukopenia reflected her sensitivity to PAS. It is more difficult to explain the leukocytosis following the test dose of PAS. This could hardly be considered a sign of allergic tolerance when she exhibited so many signs of reaction to the drug.

Kierland and Carr (2) reported four cases in which oral PAS caused severe dermatitis; two of these showed concurrent fever. One of their patients also reacted to sodium salicylate but not to para-aminobenzoic acid. This was considered evidence of sensitivity to the salicylic acid radical of PAS. In contrast, the present patient did not react to sodium salicylate or para-aminobenzoic acid. Hence, one might surmise that her sensitivity depended on exposure to the intact molecule of PAS rather than to a fractional part of that molecule.

As a rule the cerebrospinal fluid is not examined unless some disease of the central nervous system is suspected. When a drug used as commonly as is PAS in the treatment of tuberculosis produces changes suggesting meningitis, the problem of differential diagnosis is considerably complicated. Further knowledge regarding increased cellularity of the cerebrospinal fluid might be obtained if more lumbar punctures were done on patients exhibiting febrile reactions to PAS.

SUMMARY

A case is reported in which oral para-aminosalicylic acid produced fever, headache, stiff neck, and an increased cell count in the cerebrospinal fluid.

SUMARIO

Reacción al Acido Para-Aminosalicílico Simulando Meningitis Tuberculosa

En el caso descrito, el ácido para-aminosalicílico administrado por vía oral produjo fiebre, cefalalgia, tortícolis e hiperglobulia en el líquido cefalorraquídeo.

RESUME

Réaction à l'acide para-aminosalicylique simulant une méningite tuberculeuse

Il est rapporté une observation dans laquelle l'acide para-aminosalicylique (PAS) administré par voie buccale a déterminé les phénomènes suivants: fièvre, céphalée, raideur de la nuque et augmentation du nombre de cellules dans le liquide céphalo-rachidien.

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AN ANOMALOUS TRACHEAL BRONCHUS TO THE RIGHT UPPER LOBE[1,2]

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(Received for publication July 23, 1951)

INTRODUCTION

Anomalies of the lung and bronchial tree are being diagnosed with increasing frequency as a result of advances in thoracic surgery, increased use of bronchoscopy and bronchography, and further study and classification of the bronchi and their distribution.

The present report deals with a patient in whom a supernumerary tracheal bronchus to the right upper lobe was diagnosed by bronchoscopy and bronchography.

CASE REPORT

W. L., a 55-year-old male, was diagnosed in January 1950 as having pulmonary tuberculosis and was admitted to this hospital August 3, 1950. A chest roentgenogram revealed far advanced tuberculosis with multiple cavitation in the left upper lobe. As part of the preoperative preparation for a projected left thoracoplasty, a bronchoscopy was performed on August 29, 1950. The report read, in part, "There is a 75 per cent stenosis of the right main bronchus which, however, shows no mucosal changes or distortion." Inasmuch as the right lung was normal roentgenographically and by physical examination, another bronchoscopy was performed on September 20, 1950, which revealed normal findings. There was no evidence of the previously described stenosis.

Bronchography: Lipiodol bronchography was performed January 5, 1951. This revealed an anomalous accessory bronchus (figures 1 and 2) arising from the right wall of the trachea and supplying a portion of the right upper lobe. As can be seen from the accompanying illustrations (figures 1 and 2), the major right upper lobe bronchus occupied its normal position, as did the other major bronchi. The right upper lobe bronchus gave off its three normal branches. The anomalous bronchus arose 5 cm. above the carina and was 2 cm. in length and 0.75 cm. in diameter. It supplied a small segment of the medial anterior portion of the right upper lobe and apparently presented the arborization normally found with other bronchi, including an alveolar filling pattern.

Apparently upon the first bronchoscopic examination the bronchoscope was advanced along the right wall of the trachea so that the accessory bronchus was mistaken for the right main bronchus.

COMMENT

Foster-Carter (1) classifies anomalies of the lungs and bronchi into three groups: abnormalities of lobation, abnormalities of the bronchi, and abnormalities of the parenchyma. He further subdivides bronchial anomalies into (a) supernumerary bronchi, (b) displaced bronchi (when the bronchi are normal in number but abnormal in position), and (c) congenital cystic disease.

Supernumerary bronchi arising from the trachea are quite rare. In all of the reported cases, the accessory bronchus arose from the right side of the trachea above the carina and entered the right upper lobe or an accessory lobe in intimate relation with the right

[1] From the Tuberculosis Service, Veterans Administration Center, Dayton, Ohio, and the College of Medicine, University of Cincinnati.

[2] Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

upper lobe. Bremer (2) in a series of eighty human embryos found supernumerary tracheal buds in three instances but the infrequency of tracheal bronchi suggests that these buds may be resorbed in most cases.

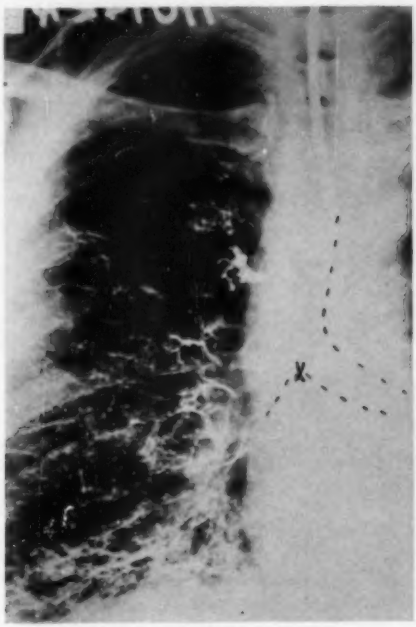

FIG. 1. Postero-anterior roentgenogram of chest, retouched to show location of carina, left tracheal wall, and left main bronchus. The anomalous bronchus can be seen 5 cm. above the carina and entering the right upper lobe. While it seems to narrow rapidly, normal arborization is present.

Overholt and Langer (3) state:

One encounters from time to time anomalies which give freak bronchial patterns. They are recognized after bronchoscopy or complete bronchography. An accessory right upper lobe bronchus may be found which takes its origin from the lateral wall of the trachea slightly above the orifice of the right main stem.

The only detailed anatomical description we could find of a case of this sort was reported by Monk (4). This was based upon an autopsy specimen in which an abnormal tracheal bronchus arose from the right posterolateral aspect of the trachea 1.5 cm. above the carina. The bronchus was 1.5 cm. in length and 1.0 cm. in width and entered the right upper lobe to supply apical and subapical bronchopulmonary segments. In this case, however, the right upper lobe bronchus had only "pectoral" and "axillary" branches (instead of the usual three main subdivisions: apical, posterior, and anterior (5)) and so the tracheal bronchus would be classified by Foster-Carter as "displaced" rather than

supernumerary. The right pulmonary artery had three branches, two going to the upper lobe. The extra ascending vessel supplied the area of the abnormal bronchus. The pulmonary veins were normal.

FIG. 2. Left anterior oblique view, retouched to show the location of the carina. The origin from the right anterolateral tracheal wall is illustrated.

McEwen (6) reported a case of a patient who had had a left lower lobectomy for bronchiectasis. A subsequent lipiodol bronchogram revealed an accessory bronchus arising from the right wall of the trachea a short distance above the carina. It appears to supply a small additional lobe lying medially and extending from the level of the lower border of the fifth dorsal vertebra to the level of the upper border of the third. The lung tissue is abnormal and appears to be composed of a number of small cysts, of varying size, which communicate with the bronchus.

As the author himself points out, however, "That the bronchus does supply a separate lobe is not proven beyond doubt."

Bronchoscopic examination revealed the supernumerary orifice on the right tracheal wall, one inch proximal to the carina. In his discussion McEwen refers to two other cases. In one of these the accessory pulmonary tissue derived its blood supply from the right subclavian artery.

Brock (7) in his "Observations on the Anatomy of the Bronchial Tree" discusses supernumerary and anomalous bronchi and states:

. . . the commonest frankly anomalous or supernumerary bronchus is seen in connexion with the right upper lobe. It springs from the lateral aspect of the right main bronchus 1.5 to 2.5 cm. above the upper lobe stem or even slightly higher, in which case it arises from the lowest part of the right side of the trachea.

Brock had seen four examples of this supernumerary bronchus; only one of these was diagnosed clinically (by bronchography), the other three being encountered during dissection.

The case of the present report is one of a supernumerary bronchus since the usual three segmental branches of the right upper lobe bronchus can be visualized on the bronchogram. It is not known whether an abnormal additional paratracheal lobe was also present or whether the area of the right upper lobe supplied by the bronchus was segmentally or otherwise separated from the adjacent right upper lobe. Brief mention should be made, however, of the fact that such a bronchus is never related to another anomaly, the azygos lobe, also located in this portion of the chest. The azygos lobe is an anomaly of the azygos vein rather than of the lung (8) and the separated azygos lobe is supplied by the usual bronchial branches (7). Foster-Carter (1) presents detailed anatomical studies of the bronchial and vascular supply to the azygos lobe, but in none was any bronchial anomaly or tracheal bronchus discovered.

The bronchial anomaly in the present case is of more than academic interest for three reasons: First, if diagnosed upon bronchoscopy as stenosis of the right main stem bronchus (as in the present case), serious errors in therapeutic judgment may follow. Fortunately, the correct diagnosis was made by means of a second bronchoscopy and bronchography. Second, during surgical removal of a lobe to which anomalous bronchi are distributed, difficulty may be encountered in identifying normal structures if the anomaly is not properly evaluated. Third, abnormal bronchi may be associated with additional or anomalous pulmonary arteries (4, 6) which may also raise surgical problems during resection.

It will be noted that the location of this bronchial orifice is such that a routine bronchogram may not reveal the lesion. If the lower lobes only are filled, the oil will not flow into the abnormal orifice. If a bronchogram is done using a catheter which was introduced directly into the right main bronchus and then into the left main bronchus, there still may be no filling of the anomalous structure since the only opening is in the trachea rather than in either of the main bronchi.

SUMMARY

An anomalous tracheal bronchus to the right upper lobe is reported and the bronchoscopic and bronchographic features of this anomaly are described.

SUMARIO

Bronquio Traqueal Anómalo Unido al Lóbulo Superior Derecho

El bronquio traqueal anómalo aquí descrito iba al lóbulo superior derecho. Después de describir las características broncoscópicas y broncográficas de la anomalía, se discuten ciertos puntos de importancia clínica.

RESUME

Présence d'une bronche trachéale anormale au niveau du lobe droit supérieur

La présence d'une bronche trachéale anormale au niveau du lobe droit supérieur est rapportée.

Les caractères bronchoscopiques et bronchographiques de cette anomalie sont décrits.

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Editorial

Lymph Node Tuberculosis and Its Treatment in Accessible Nodes

Lymph node tuberculosis appeared in the literature in the fifth century when Clovis, king of the Franks, applied the "king's touch" for the treatment of scrofula (tuberculous cervical lymphadenitis). Thus was inaugurated a form of therapy which continued in vogue for more than 1,200 years until it was finally abandoned early in the eighteenth century after Queen Anne had used it unsuccessfully on Samuel Johnson. Thereafter, various forms of medical and surgical treatment proved equally ineffective until shortly after the turn of the present century when Dowd (J.A.M.A., 1916, *67*, 499) revised the surgical technique and produced results which were excellent from the cosmetic as well as the clinical aspects. Other surgeons, notably Hanford (Nelson's *New Looseleaf Surgery*, Thomas Nelson & Sons, New York, 1933, p. 118 A) were equally successful. However, in the early nineteen twenties the incidence of tuberculous infection of accessible lymph nodes (neck, axilla, and groin) declined markedly, due, for the most part, to the better treatment of tuberculosis in general. Now, although there is plenty of it about, it seems to have been forgotten that lymph node tuberculosis still presents its own special problems which must be solved by the best methods available whether they are recent or of long standing.

Lymph nodes are involved early in the course of tuberculosis, as in the primary complex of the pulmonary disease of childhood; and it is logical to assume that the origin of infection in the peripheral nodes also lies in the area drained by them. Medlar (personal communication) believes that this is so although the focus cannot always be found and may have healed. Grady (Am. Rev. Tuberc., 1951, *63*, 526) reports a series of cases of skin tuberculosis, many of which showed involvement of the regional nodes. My own experience has been similar to his. All cases cannot be explained on this basis, however, and undoubtedly hematogenous infection plays an important role, as stated by Lincoln (Am. J. Med., 1950, *9*, 623). The predilection for the neck in hematogenous infection is probably on the basis of lowered tissue resistance due to previous infections of a different nature.

The human type of tubercle bacillus is responsible for all but an occasional case of lymph node disease. There is a widespread impression that most of the cervical lymph node infections are due to the bovine organism, but in 1908 Park and Krumwiede (J. Med. Research, 1910, *23*, 205) examined a considerable number of tuberculous nodes removed from the necks of children in New York City and found that more than 70 per cent were infected by the human type of tubercle bacillus. Since that time, rigid government inspection, at least in this country, has practically eliminated tuberculosis from dairy herds and with it the bovine infection in humans. Abroad there is still a certain amount of bovine tuberculosis although the incidence is probably decreasing.

Since the treatment employed is determined by the pathologic condition

present, certain features of the pathology of tuberculosis in general and lymph node tuberculosis in particular should be emphasized. Tuberculosis usually commences as a generalized infection with more or less systemic reaction. The local manifestations which follow are characterized by an acute inflammatory process, the exudative phase, and, when lymph nodes are involved, this is manifested by swelling, tenderness, and, often, local heat. This is followed by tubercle formation, caseation necrosis, and cold abscess. The process may be halted by fibrosis or calcification at any point but there is always a perilymphadenitis present which binds the involved nodes to one another and to the surrounding tissue. Liquefaction starting within the node usually perforates slowly into the surrounding tissue, and the resulting cold abscess has a wall of fibrous and tuberculous granulation tissue. When the pathologic process is halted by fibrosis, the encapsulated caseous material and tuberculous pus harbor organisms for an indefinite time which are capable of reactivating the infection, just as happens in pulmonary caseous foci. In this connection it must be remembered also that tuberculous lymphadenitis may be only the local manifestation of a general infection with active foci elsewhere, which must always be sought.

The ideal treatment for tuberculosis, regardless of its site, is surgical eradication if this can be accomplished, since it is better to remove an infection than to encapsulate it. However, the application of surgical excision to accessible lymph nodes seems to be a controversial subject. It is hard to understand why this is so. In the first place, surgical removal of tuberculous nodes was highly successful thirty-five years ago and there is nothing in the present status of the disease or in modern surgical skill which should alter the case. In the second place, excisional therapy for pulmonary tuberculosis is a widely accepted and efficacious form of treatment and the same principle applied to lymph node tuberculosis should be even more effective and certainly less hazardous. Nevertheless, cases for lymph node surgery should be selected with as much care as those for pulmonary surgery; an adequate surgical procedure should be employed; and full use should be made of the various adjunctive treatments, many of which were not available to the surgeons of the preceding generation.

The same principles apply to the selection of cases for lymph node surgery which apply to the selection of other cases of tuberculosis for surgery. The patient should be a "good chronic." Operation should not be undertaken in the presence of an acute exudative infection nor should lymph node surgery be attempted in the presence of active foci elsewhere except under certain definite conditions. The general condition of the patient should be sufficiently good to permit a long operation.

The surgical procedure has recently been described by the writer (Surg., Gynec. & Obst., 1948, *87*, 719) and is essentially the same as that employed by Dowd. Incisions are made in, or parallel to, natural creases so that the scar will be inconspicuous, an important consideration since most of the involved nodes are in the neck. All the diseased nodes should be removed and, as the process is always more extensive than it appears to be before operation, this requires careful, sharp dissection which takes time and persistence. Cold abscesses are dis-

sected out, not simply curetted and drained, and the underlying nodes responsible for them are removed. Contamination of the wound with tuberculous pus will not compromise the result and the wound need not be drained on this account. All of the important structures are preserved except, rarely, the mandibular branch of the facial nerve which may be damaged, although often only temporarily.

In this respect the operation differs materially from the radical neck dissection for malignancy. In that operation, important structures are sacrificed to ensure complete removal of malignant cells. With tuberculous nodes it is better to preserve the structures and perform another operation if additional nodes become swollen, something which is rarely successful where cancer is involved. Obviously, the extent of the disease has a bearing on the likelihood of reoperation but, nevertheless, reoperation is the exception and not the rule. In the end result the scar is inconspicuous, deformity is absent, and the disease is controlled.

Careful selection of cases implies that surgery is not applicable to all cases. In some it is inadvisable and in others it is unnecessary. In still others it requires the help of other forms of therapy. The general measures of rest, adequate diet, and hygienic surroundings should be employed in all cases. Certainly, they are the first consideration in the acute form of the disease, and in the mild forms no other treatment may be needed. For the most part, however, this form of treatment is chiefly an adjunct to more specific forms.

Roentgen irradiation has long been a treatment for tuberculous nodes. By producing fibrosis it acts to supplement nature's effort to encapsulate the disease. It cannot destroy *Mycobacterium tuberculosis*; it cannot remove caseation; it cannot cause the absorption of tuberculous pus and may even hasten its appearance. Furthermore, the incidence of activation of tuberculosis in other parts of the body after irradiation of lymph nodes has been observed too often to consider it a mere coincidence, even though the relation cannot be definitely proved. Its use should be limited to the treatment of sinuses after the caseation has largely disappeared and in that stage of the infection between the exudative phase and the establishment of caseation. It can then be a valuable adjunct.

Chemotherapy, notably with streptomycin and para-aminosalicylic acid (PAS), is a fairly recent and valuable addition to the therapy of tuberculous lymphadenitis but the wave of enthusiasm it has evoked in this connection is not justified by the results. Antimicrobial drugs have their chief effect on the early, exudative form of the disease and have little value in the treatment of caseous foci or cold abscesses. However, chronic tuberculous sinuses do respond well to antimicrobial therapy. In all cases the microorganisms must be sensitive to the drug; and in all cases prolonged use of the drug will result in drug-resistant microorganisms. The concomitant use of PAS will retard this resistance but cannot prevent it. Hence, antimicrobial drugs should be employed for clear indications or when nothing else is available, lest an occasion arise later when the drug is urgently needed and proves to be ineffectual because the microorganisms are resistant. One of the prominent indications for the use of chemo-

therapy is in conjunction with surgery as a prophylaxis against spread, particularly in those instances when surgical removal of tuberculous nodes must be undertaken in the presence of active foci elsewhere. When the danger of spread is past, antimicrobial therapy should be discontinued.

The most recent adjunct is the streptokinase-streptodornase combination of proteolytic enzymes developed by Tillett, Sherry, and Christensen (Proc. Soc. Exper. Biol. & Med., 1948, *68*, 184) which has been used by Hazelhurst (Surgery Forum, Clinical Congress of American College of Surgeons, W. B. Saunders, Philadelphia, 1951, p. 439) in the treatment of cold abscesses. Peripheral lymph nodes may become involved in the course of pulmonary tuberculosis and they are prone to break down into cold abscesses which have a bad effect on the pulmonary disease. Because of the pulmonary focus, excision of the nodes is inadvisable and simple drainage of the cold abscesses usually results in chronic draining sinuses. However, the use of these enzymes in the cold abscess cavity after wide drainage has been established produces a biologic debridement of the caseous material, permitting the growth of healthy granulations and closure of the sinus. The effect of such a procedure on the pulmonary focus is frequently dramatic. Later, when the condition of the patient warrants, excision of the nodes can be performed.

In conclusion, it is to be remembered that tuberculosis in peripheral lymph nodes, particularly the neck, is still frequently encountered by the physician. Due to the location of these infected nodes, they lend themselves naturally to surgical excision. Care should be taken in selecting cases for operation and, when operation is inadvisable or unnecessary, other forms of treatment are available which can also be used as an adjunct to surgery. Good results can be expected if the limitations as well as the potentialities of the various forms of therapy are heeded.

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Notes

INFLUENCE OF METHODS OF PREPARATION ON THE VIABILITY OF BCG VACCINE

In a recent note published in THE AMERICAN REVIEW OF TUBERCULOSIS (1951, *63*, 714) referring to the viability of BCG vaccine and to factors responsible for the rapid destruction of bacilli in standard vaccines, Doctor Fenner suggests that factors involved in the pellicle growth and grinding of the bacteria in a ball mill are perhaps the most important. The author stresses the higher degree of viability of the vaccine grown in liquid Tween-albumin medium so that those responsible for BCG production may be encouraged to take advantage of the highly durable product obtained by dispersed growth of BCG in Dubos liquid medium.

Investigations carried out by the present author at the Institute of Hygiene of Geneva (Grasset and Bonifas, Bull. Acad. suisse Sc. méd., 1951, *7*, 71) have led him to share the opinion that the viability of BCG vaccine appears to be linked, in a large measure, to the method of cultivation and process of preparation of the vaccine. A short account of the results of this work may, therefore, be of interest to the readers of THE AMERICAN REVIEW OF TUBERCULOSIS.

BCG vaccine suspensions of various origins from the Pasteur Institute, Paris; State Serum Institute, Copenhagen; and Institute of Hygiene, Geneva, all prepared according to the usual method, i.e., pellicle growth on Sauton medium, followed by grinding, have been submitted to examination by means of the electronic microscope. In all of the vaccines, the investigations have disclosed evidence that an important proportion (up to 40 to 60 per cent of bacillary units) showed considerable morphologic and structural alterations, such as ruptured, collapsed bacilli and ghost forms emptied of their protoplasm.

Such structural alterations are incompatible with the viability of these microorganisms, which are therefore deprived of immunizing power.

A far greater degree of structural integrity is observed in the case of bacilli constituting the BCG vaccinal suspension obtained from dispersed cultures grown in modified Dubos medium in which bovine plasma albumin has been replaced by heated human albumin. This technique necessitates no process of dissociation of the vaccinal suspension, the dispersion of the latter being realised during the growth by the presence of Tween in the medium.

The high degree of viability of the homogeneous vaccine prepared in Dubos medium finds its corollary in the high allergic power of the BCG vaccine, as demonstrated in animals immunized with the latter, as well as in the high bacterial counts of cultures obtained on Löwenstein medium, planted with progressive dilutions of vaccine after various periods of storage.

The observation of these facts, confirming the results of Dubos, brings, in the present writer's opinion, further evidence of the valuable advantages of the method for the preparation of a BCG vaccine of high activity (Grasset and Bonifas, Rev. suisse Path. gén. et bact., 1950, *13*, 642).

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PRESERVATION OF CULTURES OF M. TUBERCULOSIS BY FREEZING

During the course of a long-term study of antimicrobial therapy of tuberculosis it became necessary to preserve a large number of strains of *M. tuberculosis* for simultaneous testing of their sensitivity to the various drugs employed in the therapy. Although conventional techniques for preservation of cultures had proved satisfactory, they were sufficiently time consuming and cumbersome to make a simpler technique desirable. The method to be described, that of preservation of cultures in the frozen state, has been used in this laboratory since early in 1948 and has proved entirely satisfactory. Similar methods have been reported for use with bacterial species other than *Mycobacteria* and mention had been made previously of similar techniques employed with *Mycobacteria* (Heckly, R., Am. Rev. Tuberc., 1950, *62*, 99; Steenken, W., and Wolinsky, E., in *Streptomycin: Its Nature and Practical Application*, by Selman A. Waksman, The Williams & Wilkins Company, Baltimore, Md., 1949.) Inasmuch as the previously reported experience is limited and the technique appears not to have been widely adopted, it seemed of interest to record the experience gained in this laboratory.

Following primary isolation of the individual strains of tubercle bacilli, subcultures were made into liquid oleic acid-albumin medium and the subcultures incubated at 37° C. for seven days. At this time a 1 cc. aliquot of the culture in oleic acid-albumin medium was transferred to an agglutination tube (10 cm. by 1.5 cm.) and frozen by immersion in a dry ice and alcohol bath. The stopper was then paraffined and the tube placed in a suitable container in the cold box at minus 20° C. (Customarily, three replicate tubes were also prepared at this time but for simplicity of description only one need be considered here.) When it was desired later to test a particular strain, the culture was allowed to thaw at room temperature and, after thorough mixing, appropriate subcultures were inoculated.

Regular use of this method has been made for more than three years and it has proved to be a simple and useful procedure. To provide an example of its suitability prior to preparation of this note, replicate tubes of the first 50 strains so treated were chosen for testing of viability. These strains had been maintained in the frozen state for periods ranging from 865 days to 1,264 days (median, 1,033 days; mean, 946 days). Each culture was allowed to thaw at room temperature and, after thorough mixing, 0.1 cc. was inoculated over the surface of a solid culture medium (oleic acid-albumin agar) in a Petri dish. These cultures were then incubated at 37° C and examined weekly.

Of 50 strains thus examined, colonies of tubercle bacilli were demonstrable in the subcultures of 49 at the end of one week of incubation. One specimen was sufficiently contaminated with fungi so that the presence of *Mycobacteria* could not be detected by this procedure. The morphology of the colonies of tubercle bacilli was slightly altered from that characteristically ascribed to virulent tubercle bacilli in that cords were somewhat finer and longer. On stained smears of these colonies it was noted that large numbers of the bacilli were nonacid-fast. The first subcultures from these specimens, however, appeared to have the same rate of growth, the same colony morphology and staining characteristics as the stock laboratory strain H37Rv.

At the time of thawing of the test strains, in addition to the inoculation of the solid medium, 0.4 cc. aliquots were inoculated directly into liquid oleic acid-albumin medium and into Tween-albumin medium. As might be expected, growth was not as readily discernible in the liquid medium, but in 48 of the 50 strains thus examined macroscopically visible growth was present in the two liquid media at the end of two or three weeks' in-

cubation. Contamination of two strains accounted for the two failures to recover tubercle bacilli in the liquid medium.

Three additional strains of tubercle bacilli which had been maintained in the frozen state for approximately three years were allowed to thaw and then were directly inoculated subcutaneously into guinea pigs. All 3 strains produced characteristic tuberculous lesions in the guinea pigs.

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A NEW METHOD OF BCG PRODUCTION

The method to be reported is one in which the bacilli do not leave their container from the time they are planted until the time they are dispensed, thus eliminating, to a great extent, exposure to the air and possible contamination from the outside. In addition, the simplicity of the method and the saving of time, glassware, and apparatus make this method a practical one, especially since the yields are four to six times as great per container as they are when the standard method is followed.

Special apparatus needed: (*1*) One special funnel and lid for the stainless steel ball bearings: diameter of the funnel 5½ inches, over-all length 12 inches, the diameter of the stem being 1 inch. An obturator, at base of funnel, attached to a steel wire, is used for releasing the balls. The lid contains two openings, one for the steel wire and one for pouring, the latter being 1 inch in diameter (figure 1E). (*2*) Two metal racks to support culture flasks (figure 1B, 2F).

Other standard equipment needed: Bacterial culture flasks of low form, 2,500 ml. capacity[1]; stainless steel balls ¼ inch in diameter, 9 lbs. for each flask[2]; dispensing and suction jars (figures 1F and ID, respectively), rotary with verticle table, capable of developing 30 revolutions per minute (figure 2A); stainless steel tubing ¼ inch in diameter (figures 1C, 2B).

Production of Vaccine

The vaccine is made from 12- to 14-day-old cultures grown on Sauton in the bacterial flasks mentioned above containing 500 ml. of medium. At this time, the entire surface is covered by an undulating membrane that extends onto the sides of the flask. In removing the flask from the incubator, it is tilted immediately so that the veil adheres to the base of the flask, thus anchoring the surface growth. The flask is then rotated to a vertical position and placed in a special rack, as shown in figure 1B. By means of a suction tube the entire fluid is aspirated (figure 1D). The flask is then returned to its horizontal position and the funnel containing the steel balls (figure 1E) is removed from its cloth wrappings and placed into the mouth of the flask. The balls are released by pulling on the steel wire which disengages the obturator. At this point, 40 ml. of distilled water are added through the pouring aperture.

First, grinding is done by hand for a minute or two until the culture is well dispersed. The flask is then placed on a vertical table (figure 2A), and rotation in this position at 30 r.p.m. is continued for fifteen minutes. After the grinding, approximately 300 ml. of Sauton's medium diluted to one-quarter strength are added by means of a dispensing jar and tube, as shown in figure 1F. In this way, there is no exposure of the fluid to the air

[1] Corning Glass Company, Corning, New York, No. 4422.

[2] Hoover Ball and Bearing Company, 520 N. Michigan Avenue, Chicago, Illinois.

FIG. 1. Culture flask (A) in horizontal position on special rack (B), curved, stainless steel tube attached to gauze and cotton stopper and paper skirt (C). This is connected to a suction flask (D) which, in turn, is connected to a suction pump.

Special funnel, made of stainless steel, for the stainless steel balls (E), dispensing jar (F) on shelf contains diluent S/4 attached to a straight tube, stopper, and paper skirt (G).

FIG. 2. To the right, rotary (A) with clamps for grinding the vaccine. To the left, dispensing setup.

Note tube (B) in flask extending to bottom. It is held in place by stopper and skirt (C). Side arm (D) is to start flow of vaccine. Also note needle and glass bell (E) used for filling ampules. The rack (F) fixes flask.

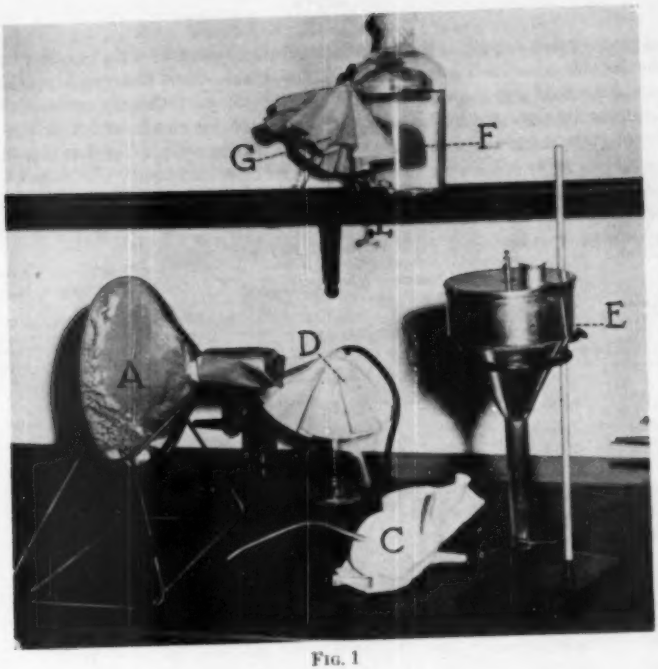

FIG. 1

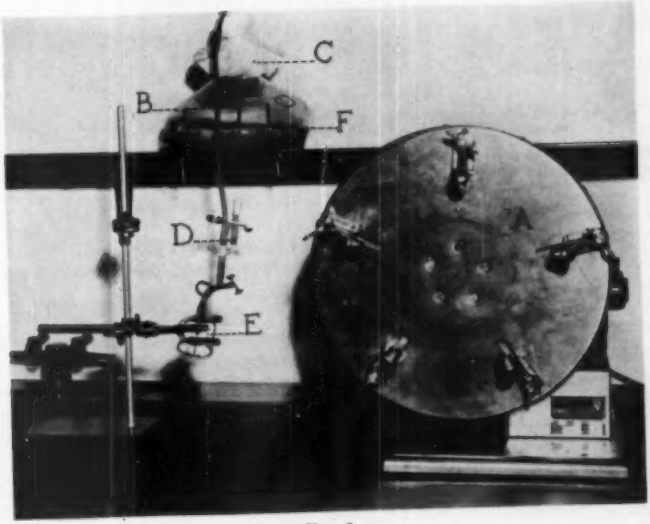

FIG. 2

since a gauze-cotton stopper at the base of the tube fits tightly over the mouth of the flask, and the dispensing jar itself is stoppered with gauze and cotton. Rotation is continued by hand until the fluid and the vaccine are well mixed, at which time approximately 2 or 3 cc. of vaccine are aspirated by means of a stainless steel tube and a 30 ml. syringe. From this sample, the concentration is determined by comparing a diluted sample to a standard

TABLE 1

Viability and Tuberculin Conversion in Guinea Pigs and Newborns of BCG Vaccine

(17 Consecutive Lots)

NUMBER OF VACCINE	DATE	VIABILITY STUDIES		TUBERCULIN CONVERSION			
				Animals†		Humans‡	
		Age of Vaccine	Growth*	Number	Number Positive	Number	Number Positive
		days					
895K	6–29–50	8	10^{-10}	2	2	6	6
896K	7–6–50	1	10^{-10}	2	2	11	11
896L	7–13–50	1	10^{-9}	2	2	10	10
		8	10^{-9}				
890H	7–20–50	1	10^{-9}	2	2	4	4
891H	7–27–50	5	10^{-9}	2	2	9	9
898K	8–3–50	5	10^{-10}	2	2	5	5
		8	10^{-10}				
892K	8–10–50	1	10^{-10}	2	2	11	11
892K	8–17–50	4	10^{-9}	2	2	8	8
892H	8–24–50	1	10^{-9}	1	1	7	7
900K	8–31–50	5	10^{-10}	2	2	11	11
900L	9–7–50	1	10^{-10}	2	1	9	9
		15	10^{-9}				
901K	9–14–50	1	10^{-9}	2	2	9	9
		5	10^{-9}				
901K	9–21–50	1	10^{-10}	2	2	8	8
		8	10^{-10}				
902K	9–28–50	5	10^{-9}	2	2	12	12
902L	10–5–50	5	10^{-9}	2	2	10	10
903K	10–12–50	4	10^{-8}	2	2	9	8
903L	10–19–50	5	10^{-8}	2	2	9	9

* In Youmans' fluid medium plus ablumin.

† Vaccinated by one puncture and tested one to four weeks later.

‡ Vaccinated by multiple puncture disc (36 punctures) and tested four to eight weeks later.

BCG vaccine suspension[3] or turbidimetrically in a Coleman spectrophotometer. The desired concentration is prepared by adding more diluent. After mixing well by hand, an aspiration tube (figure 2B) is placed in the flask, touching the bottom, and secured there firmly by the cotton-gauze covered stopper at the base of the tube which fits onto the mouth of the flask and by the paper skirt which is tied around the mouth of the flask (figure 2C).

Flow is initiated by aspirating with a 30 ml. syringe at the side arm (figure 2D) of this

[3] Hopkins' tube method verified turbidimetrically.

tube. A clamp, as indicated, controls the flow of vaccine through an injection-type needle (figure 2E). The ampules are then filled in the usual manner.

The table gives the results of 17 consecutive lots of vaccine made by this method in regard to viability and conversion to tuberculin hypersensitivity in animals following one puncture and in humans following 36 punctures by the multiple-puncture disc. For animals, 10 mg. of Old Tuberculin were used and readings were made at 24 and 48 hours. For humans, 1 mg. of Old Tuberculin was used and readings were made after 48 hours. An induration of 10 mm. or more was considered positive.

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Books

THE PRACTICE OF MEDICINE. Fifth edition. By Jonathan Campbell Meakins. Pp. 1,558, The C. V. Mosby Company, St. Louis, 1950, Price, $13.50.

During the fifteen years which have elapsed since publication of the first edition, Meakins' text has become established as one of the most popular and widely read books in the field of internal medicine. A careful perusal of the fifth edition reveals that all of the features which contributed to its earlier success have been retained. Outstanding among these features are the clear, concise style of presentation, the conservative approach to controversial issues, the excellent index, and the large number of illustrations.

Meakins' book will inevitably be compared with texts which are written by several dozen authors, each carefully selected on the basis of special interests and experience which qualify him to write an authoritative and up-to-date account of one, or at most a few, diseases. Such a person might presumably be better able to evaluate controversial subjects and to present newer concepts and techniques than one who is attempting to encompass the entire field of medicine. To evaluate this point, the reviewer has carefully compared many portions of Meakins' book with similar sections of Cecil's *Textbook of Medicine*. The presentation is in many instances equally authoritative and up to date in both books. In other instances, however, the specialists' more intricate knowledge of a given subject makes Cecil's book considerably more current and factual. An example may be cited. Meakins says of hemochromatosis that: "No specific cause is known and its fundamental nature is uncertain, but there would appear to be a defective excretion of iron." Evidence accumulated during the past several years indicates, however, that there is no abnormality in the excretion of iron in patients with hemochromatosis; the defect appears rather to be one of increased absorption. This and similar examples have convinced the reviewer that the growing complexity of medicine has made it virtually impossible for a single author to present the subject matter with the same timeliness and authority that can be achieved by a group of experts.

Despite this limitation, one is repeatedly impressed with the many superior qualities of Meakins' text. The excellent basic descriptions of disease and the carefully chosen illustrations make it an excellent introduction to internal medicine for the medical student. Many features, such as the charts and description of serum therapy in pneumococcal pneumonia, have been retained chiefly for historical interest and add to the general understanding of the subject. Tuberculosis is well described, and the newer developments in chemotherapy are presented as well as is possible in this type of book. All in all, one can safely predict that this text will continue to enjoy well-deserved success and popularity with both medical students and practitioners.

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HUMAN GENETICS. Two volumes. By Reginald Ruggles Gates. Pp. 1,518, with illustrations, The Macmillan Company, New York, 1946, Price, $15.

The author's stated purpose is "to make available present knowledge" of human genetics. This purpose he accomplishes as nearly as could be hoped by abstracting material from more than 5,500 papers and books covering such diverse subjects as the human chromosomes, hereditary diseases of the nervous system, and race mixture. As a scientific text,

the book might be criticized for its many expressions of unorthodox opinion. As a bibliography, its usefulness is increased by logical organization and by an excellent ninety-page index for subjects and for authors.

The first chapter is a general introduction; the next three illustrate genetic principles in man. Of the remaining twenty-seven chapters, twenty-one deal chiefly with medical conditions and six deal with inheritance of normal variations. Most subjects are accorded space in proportion to the genetic literature available and those most extensively studied occupy entire chapters; for example, blood groups, cancer, allergy.

In the introduction the author illustrates the standard symbols for pedigree diagrams and makes a plea for their wider acceptance, especially in medical publications. He also suggests that many uncommon conditions which are usually considered nonhereditary because they show little or no familial tendency probably represent dominant mutations or the out-cropping of recessive genes. Current genetic literature tends to support this concept. In the second chapter, on *General Principles*, he explains most of the concepts and terms of modern genetics but other geneticists have disagreed with some of the explanations. (These matters are treated with accuracy and detail in the recent book by Curt Stern, *Principles of Human Genetics*.)

Gates does not attempt to cover the rather specialized field of population genetics. Thus, he neglects the concept of equilibrium between mutation and selection. The importance of this is being accentuated both by advances in medical science and by increasing exposure of humans to ionizing radiation.

After the introductory chapters, the author generally refrains from expressing his own opinions or from criticizing the papers he reviews. With a few exceptions, topics and chapters are not summarized. Occasionally he draws conclusions which were not intended by the authors or with which most geneticists would disagree. This is best illustrated by his frequent inference of genetic linkage between traits on the basis of common association between them.

Special attention is given to tuberculosis in the chapter on "Constitution, Body Build and Susceptibility." In six pages he reviews some of the literature on constitutional, familial, and racial susceptibility to tuberculosis. Starting with the early study of Bulloch and Rivers (1909), he cites the work of approximately twenty-five authors, including six papers on tuberculosis in twins and three experimental studies in animals. Unfortunately, the latest references, Kallman and Reisner (1943) and Puffer (1944), receive only one sentence each. Twelve incidental references to tuberculosis are listed in the index.

The book is monumental with respect to its coverage of literature on human genetics and, together with Stern's book of similar title, ought to be available in every medical library.

GORDON ALLEN

DESIGN FOR SANATORIA. Report of the NAPT Architectural Committee (Chairman: Dr. Geoffrey Todd). Pp. 124, with illustrations, National Association for the Prevention of Tuberculosis, Tavistock Square, London, 1951, Price, 12 s. 6 d.

This book represents a brief report of the NAPT Architectural Committee on suggestions and ideas for the design and construction of sanatoriums for tuberculous patients. It contains not only advice for architectural arrangements but also for interior design and equipment. In forty short chapters the future planner is reminded of such diverse subjects as layout and construction of the building, lighting, heating, plumbing, garbage and sputum disposal, laundry, facilities for surgery, pathology, roentgenology and research,

staff accommodations, and many other things. Recommendations for the site and size of the sanatorium, elimination of noise, physiotherapy, and arrangements for tropical and semi-tropical climatic conditions will be found very useful.

Although the report stresses several times that the sanatorium represents the home of the patient for many months or for years, few original ideas were contributed by the committee to make such an institution homelike. This may be due to the fact that the reporting committee and its advisory board consisted of many specialists and public dignitaries but did not include patients. If a similar book should be written in the future, it would be advisable to include "experienced" patients in the committee. They would be able to suggest many improvements in the equipment of rooms, bathrooms, porches, and so on, which may not occur to an expert who has never spent any length of time in bed on account of illness. For instance, patients on strict bed rest might have a great deal to say about the construction of beds. Such beds are still constructed like beds for sleeping purposes and not for living. Devices for reading, writing, cleaning, shaving, calling, lighting, and many other conveniences could be suggested by patients. The cumbersome footboard should be made superfluous and replaced by a simple mechanical device to keep the blanket off the patient's feet. It would be convenient and probably economical if beds could be moved to and from the porch on rails by electricity. The report devotes only a short paragraph to W. C. 's (toilets). Patients, particularly some of those who have been treated with para-aminosalicylic acid, will have more concrete suggestions concerning the number of toilets required per patient and their accessibility. Some of the suggestions contributed by patients may prove impractical but others could be very useful to the architects and designers of equipment.

JOSEF WARKANY

SURGICAL TREATMENT FOR THE ABNORMALITIES OF THE HEART AND GREAT VESSELS. By Robert E. Gross. Pp. 72, Charles C Thomas, Springfield, Illinois, 1950, Price, $2.25.

This small book is a revised Janaury 1950 edition of an original Beaumont Lecture given in 1946 before the Wayne County (Michigan) Medical Society. The monograph covers mainly the subjects of patent ductus arteriosus, vascular ring anomalies, and coarctation of the aorta, with brief mention being given to the subject of Tetrology of Fallot and defects of the pericardium. Noticeably absent is a discussion of the other cardiac abnormalities which are now amenable to surgical treatment, especially the valvular lesions and septal defects. Furthermore, there is little mention of the recent experimental work on the abnormalities discussed and newer techniques used to correct these abnormalities. It is believed that a new revised edition should have included this newer phase of cardiac surgery. Because of these omissions and the briefness of the monograph, it cannot be recommended as a complete reference on the subject of cardiac surgery. As a brief review and lecture on the subjects (and for that it was intended), the book is well written and makes good reading.

B. W. MEYER

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DIRECTORY OF TUBERCULOSIS INSTITUTIONS IN INDIA 1950. Pp. 143, The Tuberculosis Association of India, New Delhi, 1950.

TWELFTH ANNUAL REPORT 1950. Pp. 180, The Tuberculosis Association of India, New Delhi, 1950.

MASS BCG VACCINATION IN POLAND 1948–49, with Special Reference to Statistics on Tuberculin Testing and BCG Vaccination. Pp. 134, Tuberculosis Research Office, World Health Organization, The International Tuberculosis Campaign, Copenhagen, 1950.

ELECTROKYMOGRAPHY, Proceedings of the First Conference, 1950. Pp. 215, Federal Security Agency, Public Health Service, National Heart Institute of the National Institutes of Health, Washington, D. C., 1951, Price, $0.75.

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COMMUNITY HEALTH EDUCATION IN ACTION. By Raymond S. Patterson and Beryl J. Roberts. Pp. 346, The C. V. Mosby Company, St. Louis, 1951, Price, $4.50.

THE CONFERENCE ON EUROPEAN B. C. G. PROGRAMMES. Pp. 333, The International Tuberculosis Campaign, Denmark, 1950.

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FROM DUGOUT TO HILLTOP. By Margaret R. Stewart. Pp. 233, Murray & Gee, Inc., Culver City, California, 1951, Price, $3.75.

THE PUBLIC HEALTH NURSE AND HER PATIENT. Second edition. By Ruth Gilbert. Pp. 348, Harvard University Press, Cambridge, Massachusetts, 1951, Price, $3.75.

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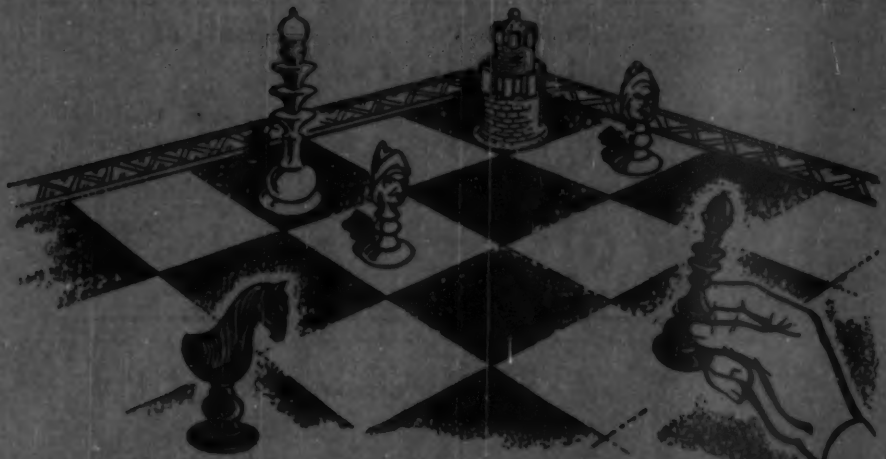

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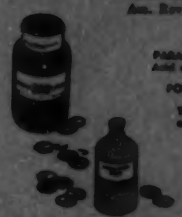

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